JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXIII WASHINGTON, D. C., JANUARY 13, 1923 NO. 2

EFFECT OF VARIOUS INORGANIC NITROGEN COMPOUNDS, APPLIED AT DIFFERENT STAGES OF GROWTH, ON THE YIELD, COMPOSITION, AND QUALITY OF WHEAT [1]

By JEHIEL DAVIDSON, *Soil Chemist*, and J. A. LECLERC, *formerly Chemist in Charge, Plant Chemical Laboratory, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

In a series of experiments reported in 1917 and 1918 [2] sodium nitrate was applied at different stages of growth. When the sodium nitrate was applied at the first stage, when the crop was about 2 inches high, an increase in yield was obtained. When applied at the second stage, at the time of heading, the yield was not affected, but a higher nitrogen content, both in the grain and straw, was obtained, and yellow-berry was practically eliminated. When applied at the third or milk stage, almost no effect was observed. The wheat used in those experiments was a soft winter wheat. The terms flinty and yellow-berry refer not to the typical qualities of wheat designated by them but only to the color of the grain.[3]

The experiment discussed in this article was undertaken with two objects in view. One was to repeat the previous experiments with a hard winter wheat in a region where the prevalence of typical yellow-berry fluctuates from year to year; the other was to determine the effect of nitrogen derived from carriers other than sodium nitrate.

The experiment was carried out on the farm of the Claassen brothers, near Beatrice, Gage County, in the State of Nebraska,[4] in the summer of 1917.

---

[1] Accepted for publication July 2, 1922.

[2] DAVIDSON, J., and LECLERC, J. A. THE EFFECT OF SODIUM NITRATE APPLIED AT DIFFERENT STAGES OF GROWTH ON THE YIELD, COMPOSITION, AND QUALITY OF WHEAT. *In* Jour. Amer. Soc. Agron., v. 9, no. 4, p. 145-154, 1917; v. 10, no. 5, p. 193-208, 1918.

[3] Since this work was completed, W. F. Gericke (ON THE PROTEIN CONTENT OF WHEAT. *In* Science, n. s., v. 55, p. 446-447. 1922; DIFFERENCES EFFECTED IN THE PROTEIN CONTENT OF GRAIN BY APPLICATIONS OF NITROGEN MADE AT DIFFERENT GROWING PERIODS OF PLANTS. *In* Soil Science, v. 13, p. 163-170. 1922) reported results which, without allowance for the peculiar conditions under which his experiments were carried out, are identical in their essential tendencies with the results just referred to. The two papers by Davidson and LeClerc,[2] which had been presented at consecutive annual meetings of the American Society of Agronomy before they were published, seem to have escaped Mr. Gericke's notice, as he asserts (Science, loc. cit.) that his results obtained with wheat, one of the plants studied, threw new light upon this protein question.

[4] The authors wish to acknowledge the valuable assistance of County Agent L. B. Rist and of the owners of the farm.

EXPERIMENTAL WORK

PLAN OF THE EXPERIMENT

The plan of the experiment is given in the following table:

Plot No.	Stage 1 (crop about 8 inches high).	Stage 2 (time of heading).	Stage 3 (milk stage).
1	Sodium nitrate (2 pounds).		
2	.....	Sodium nitrate (2 pounds).	
3	.....	.....	Sodium nitrate (2 pounds).
4	Potassium chlorid (1.8 pounds).		
5	.....	Potassium chlorid (1.8 pounds).	
6	.....	.....	Potassium chlorid (1.8 pounds).
7	Calcium nitrate (2 pounds).		
8	.....	Calcium nitrate (2 pounds).	
9	.....	.....	Calcium nitrate (2 pounds).
10	Ammonium sulphate (1.5 pounds).		
11	.....	Ammonium sulphate (1.5 pounds).	
12	.....	.....	Ammonium sulphate (1.5 pounds).
13	Sodium hydroxid (0.9 pound).		
14	.....	Sodium hydroxid (0.9 pound).	
15	.....	.....	Sodium hydroxid (0.9 pound).
16	Potassium nitrate (2.4 pounds).		
17	.....	Potassium nitrate (2.4 pounds).	
18	.....	.....	Potassium nitrate (2.4 pounds).
19	Sulphuric acid (298 cc.).		
20	.....	Sulphuric acid (298 cc.).	
21	.....	.....	Sulphuric acid (298 cc.).
22	Ammonium chlorid (1.3 pounds).		
23	.....	Ammonium chlorid (1.3 pounds).	
24	.....	.....	Ammonium chlorid (1.3 pounds).
25	Potassium hydroxid (1.3 pounds).		
26	.....	Potassium hydroxid (1.3 pounds).	
27	.....	.....	Potassium hydroxid (1.3 pounds).
28	Ammonium nitrate (0.94 pound).		
29	.....	Ammonium nitrate (0.94 pound).	

Plot No.	Stage 1 (crop about 8 inches high).	Stage 2 (time of heading).	Stage 3 (milk stage).
30			Ammonium nitrate (0.94 pound).
31	Water (25 gallons).	Water (25 gallons).	Water (25 gallons).
32		Control.	
33	Sodium chlorid (1.4 pounds).		
34		Sodium chlorid (1.4 pounds).	
35			Sodium chlorid (1.4 pounds).
36	Calcium chlorid (1.3 pounds).		
37		Calcium chlorid (1.3 pounds).	
38			Calcium chlorid (1.3 pounds).
39	Hydrochloric acid (887 cc.).		
40		Hydrochloric acid (887 cc.).	
41			Hydrochloric acid (887 cc.).
42	Potassium sulphate (2 pounds).		
43		Potassium sulphate (2 pounds).	
44			Potassium sulphate (2 pounds).
45	Magnesium nitrate (1.7 pounds).		
46		Magnesium nitrate (1.7 pounds).	
47			Magnesium nitrate (1.7 pounds).
48	Magnesium chlorid (1.1 pounds).		
49		Magnesium chlorid (1.1 pounds).	
50			Magnesium chlorid (1.1 pounds).
51	Nitric acid (078 cc.).		
52		Nitric acid (078 cc.).	
53			Nitric acid (078 cc.).
54		Ammonia water (1,400 cc.).	
55			Ammonia water (1,400 cc.).
56	Water (25 gallons).	Water (25 gallons).	Water (25 gallons).
57		Control.	

The applications of the various chemicals were calculated at the rate of 320 pounds of sodium nitrate to the acre. The nitrogen carriers were used in such proportions as to give an amount of nitrogen to the acre equal to that supplied by the application of sodium nitrate. The other elements were applied in such proportions as to give amounts to the acre equal to those supplied by the nitrogen carriers. The acids were used in amounts which were chemical equivalents of their respective neutral salts. The exact quantities of the chemicals used to the plot are shown in the foregoing table. The chemicals were applied in solution only, and 25 gallons of water were used in each case. The experiment was conducted in duplicate (series A and series B). An average of the results

of the two series was not taken, as the purpose of the work was to demonstrate tendencies rather than to obtain absolute figures.

The plots comprised 1 square rod each, with 2-foot alleys between them.

PRINCIPLES UNDERLYING SELECTION OF CHEMICALS

When flinty wheat kernels were soaked in the laboratory in a slightly acid solution they lost their brown color, and when yellow-berried kernels were soaked in a slightly alkaline solution they acquired the brown coloration characteristic of flinty grain. This suggested the idea that the color characteristics of flinty and yellow-berried grain constitute indicator reactions with alkalies and acids, as well as the possibility that the elimination of yellow-berry caused by the application of sodium nitrate was due to the residual effect of the sodium base.

The nitrogen carriers used in this experiment were accordingly of two distinct groups: Nitric acid and its salts and ammonia and its salts. The salts of the nitric acid belong to the physiologically alkaline group. Since the plants use the nitrogen of the acid radicles more readily than that of the basic elements, residues of basic radicles are left. Following the same reasoning, the salts of ammonia may be placed in the physiologically acid group. Ammonium nitrate is a physiologically neutral salt. Nitric acid would have the status of a nitrate, since it attacks the physiologically neutral salts of the soil, particularly the carbonates, thus being transformed into a physiologically alkaline substance. Ammonia, which nitrifies very readily, would have approximately the same status as nitric acid. The status of potassium nitrate would depend upon the rate of assimilation of potassium and nitrogen by the plants, which would vary with soil conditions. If these two elements were assimilated by the plants at the same rate, the status of potassium nitrate would be that of a physiologically neutral substance. Should these two elements be assimilated at different rates, its status would always be that of a physiologically alkaline substance. Should nitrogen be assimilated more readily, a residue of basic potassium would be the result. On the other hand, should potassium be assimilated more readily, the residue would be nitric acid which, as just explained, would act as a physiologically alkaline substance.

The same principle underlies the selection of the other chemicals. It was expected that the acids and alkalies would produce direct effects of alkalinity and acidity. The salts of potassium were counted on to produce physiological acidity. The physiologically neutral salts were intended to serve as controls with reference to the residual acid and basic radicles of the nitrogen carriers.

The term "residual effect" is used in this connection to signify the possible effect of residual acid or basic radicles on the soil reaction, as well as their effect on the physiological reaction of the plant tissues.

FACTORS WHICH TENDED TO DISTURB THE REGULARITY OF RESULTS

The plots were laid out in the spring just before the first application. Due to the fact that the wheat in Nebraska in that year suffered heavily from late frosts, it was impossible to obtain uniform plots. The part of the field used for series A was fairly uniform, and the plots were laid out consecutively. The section of the field which was available for series B was entirely lacking in uniformity. An attempt was made to select fairly

uniform plots for the three applications of each chemical and also for certain groups of chemicals. The more uniform plots were assigned to the nitrogen carriers. Consequently, the control plots were not true representatives of normal conditions. Deviations from the controls were not necessarily due to the treatments in the case of the tendencies which were not sharply defined but which might have been detected under normal conditions.

Another element of disturbance affecting the regularity of the results was introduced by the fact that there was no uniform transition from one stage to another. When most of the plants on the plots were headed out some had not yet reached that stage. The same thing occurred during the transition from the second to the third stage.

Moreover, a heavy rainfall, coming just as the treatment of one series was finished, interfered with the second stage application. Part of the chemicals applied to that series were thus probably washed out. The other series had to be treated when the soil was completely saturated. As a result, a portion of the chemicals which were applied in solution ran off from some of the plots. This probably accounts for part of the irregularities which will be found in the tables.

With all these disturbing factors, however, the principal tendencies resulting from the application of the various nitrogen carriers were not obscured, due probably to the fact that of the 114 plots of the experiment 54 received nitrogen. The remaining 60 plots served as controls with reference to the effects of this element. Similarly, the plots which received the nitrogen in the third stage frequently served as controls for the plots of the other two stages of the groups.

TABLE I.—*Total yield and percentage of grain from plots to which chemicals were applied at various stages of growth*

Chemicals applied.	Series A.						Series B.					
	Total yield.			Percentage of grain.			Total yield.			Percentage of grain.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
	Lbs.	*Lbs.*	*Lbs.*				*Lbs.*	*Lbs.*	*Lbs.*			
Sodium nitrate	50.2	31.1	28.5	36.9	41.9	43.7	34.7	26.5	23.4	33.3	42.3	38.2
Potassium chlorid	30.0	34.2	24.2	42.9	40.5	40.4	20.0	25.5	23.7	39.6	41.9	41.4
Calcium nitrate	45.9	34.0	23.1	37.3	41.5	42.8	35.5	25.0	22.8	35.9	38.2	38.3
Ammonium sulphate	29.5	34.5	29.3	34.5	39.3	38.6	29.5	25.4	23.5	40.1	39.4	36.8
Sodium hydroxid	27.0	25.0	21.7	36.8	45.8	40.4	27.2	28.2	21.5	42.4	39.8	42.4
Ammonium nitrate	39.8	29.5	27.5	38.5	39.4	40.7	33.5	31.8	25.4	34.8	40.2	39.5
Sulphuric acid	26.0	31.5	24.0	42.2	41.3	39.3	22.8	23.2	25.2	41.8	42.6	38.8
Ammonium chlorid	30.5	32.0	23.7	46.3	41.3	42.6	20.0	26.7	21.0	39.5	42.3	43.0
Potassium hydroxid	22.0	23.3	20.9	44.1	41.8	42.0	25.0	24.8	23.0	43.6	41.7	42.7
Ammonium nitrate	49.7	37.9	29.7	38.2	42.1	43.2	38.2	29.3	25.0	34.3	40.8	42.7
Water		26.5			43.0			23.5			44.3	
Control		24.7			43.8			23.5			41.8	
Sodium chlorid	24.1	27.5	30.0	44.0	43.1	44.1	24.7	22.8	22.9	44.2	41.9	42.5
Calcium chlorid	36.8	34.5	31.5	42.0	44.5	37.4	21.8	23.0	23.6	44.6	43.3	41.9
Hydrochloric acid	25.1	23.4	25.0	41.6	40.9	42.9	26.2	24.2	21.5	42.7	42.8	43.7
Potassium sulphate	42.7	42.1	36.7	44.4	44.5	41.9	27.5	28.5	23.0	42.7	42.3	41.0
Magnesium nitrate	34.4	30.1	24.0	40.3	42.3	45.4	33.0	24.4	21.3	37.2	41.4	42.0
Magnesium chlorid	34.1	31.2	27.1	44.4	43.1	43.8	27.0	25.0	24.1	43.8	43.8	38.2
Nitric acid	40.2	27.1	25.9	40.0	38.1	38.0	35.0	29.1	24.3	33.0	37.7	40.2
Ammonia					42.2	36.0					42.3	42.5
Water		28.5			37.7			21.5			38.0	
Control		29.0			40.7			21.7			39.8	

TABLE II.—*Percentage of yellow-berry in wheat from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.			Series B.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	10.0	1.9	19.4	7.2	5.1	17.2
Potassium chlorid	23.7	24.3	41.3	21.5	26.2	26.8
Calcium nitrate	13.5	3.2	15.0	9.7	5.2	32.0
Ammonium sulphate	15.5	3.0	33.5	5.7	1.8	11.4
Sodium hydroxid	21.4	21.4	21.8	15.9	24.4	33.2
Potassium nitrate	14.5	2.7	12.6	4.9	2.2	14.2
Sulphuric acid	33.3	28.8	42.7	21.9	29.1	13.7
Ammonium chlorid	25.8	4.5	31.7	10.0	4.4	13.4
Potassium hydroxid	26.8	24.3	20.8	9.9	8.3	7.6
Ammonium nitrate	12.4	3.1	11.7	4.1	3.1	2.3
Water		24.4			16.5	
Control		22.2			10.2	
Sodium chlorid	31.2	30.2	34.6	18.5	9.0	18.2
Calcium chlorid	31.5	31.5	34.0	22.1	12.0	12.2
Hydrochloric acid	19.2	26.1	32.4	8.1	5.5	13.2
Potassium sulphate	33.2	31.1	24.8	13.3	13.8	23.2
Magnesium nitrate	25.3	9.5	37.1	10.3	5.6	23.2
Magnesium chlorid	34.8	28.4	33.0	11.3	14.4	25.2
Nitric acid	12.1	4.2	14.7	3.7	1.8	8.5
Ammonia		1.0	13.3		0.3	7.4
Water		34.1			17.0	
Control		25.0			8.5	

TABLE III.—*Weight per bushel and weight per 1,000 kernels of wheat from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Weight per bushel.			Weight per 1,000 kernels.			Weight per bushel.			Weight per 1,000 kernels.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
	Lbs.	*Lbs.*	*Lbs.*	*Gm.*	*Gm.*	*Gm.*	*Lbs.*	*Lbs.*	*Lbs.*	*Gm.*	*Gm.*	*Gm.*
Sodium nitrate	59.8	60.1	60.5	26.8	28.8	30.0	59.1	60.5	60.5	25.8	28.7	29.6
Potassium chlorid	60.1	60.4	60.5	29.2	29.7	31.9	60.8	61.2	61.2	28.1	28.9	29.5
Calcium nitrate	59.0	59.8	61.1	24.7	27.9	30.0	60.5	60.5	61.5	26.4	27.9	28.1
Ammonium sulphate	60.1	60.5	59.8	26.0	28.4	29.7	60.5	60.8	60.5	28.0	28.8	30.3
Sodium hydroxid	60.8	61.5	60.8	28.4	28.7	28.2	60.8	61.2	59.6	29.2	29.2	27.3
Potassium nitrate	60.5	60.5	60.8	28.0	29.1	29.2	60.5	60.5	61.9	27.5	27.5	27.1
Sulphuric acid	61.1	61.1	60.5	28.6	28.6	27.5	61.1	61.2	62.2	28.5	28.6	28.2
Ammonium chlorid	60.6	61.1	61.1	28.1	28.8	27.7	60.2	58.6	61.9	28.2	27.4	27.2
Potassium hydroxid	61.5	61.9	61.1	28.2	28.8	29.0	61.9	61.2	61.2	29.1	28.9	29.6
Ammonium nitrate	60.8	61.1	62.6	27.5	28.5	29.7	60.5	60.5	61.2	27.6	28.3	28.7
Water		61.1			28.3			61.9			29.2	
Control		61.5			27.6			61.2			27.9	
Sodium chlorid	60.8	60.8	61.1	27.1	27.1	27.8	61.2	61.2	61.9	27.1	28.9	29.6
Calcium chlorid	61.9	61.9	61.1	28.9	29.3	28.6	61.9	63.2	61.5	28.5	29.7	28.8
Hydrochloric acid	61.1	61.9	61.9	27.5	29.7	27.2	61.5	61.9	60.5	29.2	29.1	27.1
Potassium sulphate	61.9	61.5	61.9	29.5	27.5	29.3	61.2	61.9	61.5	29.3	29.7	28.2
Magnesium nitrate	61.1	61.9	61.9	27.7	28.6	28.1	63.5	60.8	61.9	28.5	28.2	27.1
Magnesium chlorid	61.5	61.9	60.8	29.4	29.3	29.9	61.5	61.2	62.9	28.7	29.5	28.2
Nitric acid	62.5	61.5	61.5	28.9	27.1	27.6	62.5	60.8	61.9	28.9	28.7	28.2
Ammonia		61.1	61.9		30.1	30.1		60.8	63.2		29.5	28.5
Water		60.5			28.7			61.5			28.7	
Control		61.1			28.0			61.2			28.5	

TABLE IV.—*Correlation between protein content and weight per bushel and weight per 1,000 kernels of wheat*

Place of growth	Variety.	Year.	Protein content.	Weight per 1,000 kernels.	Weight per bushel.
			Per cent.	*Gm.*	*Pounds.*
Kansas	Crimean	1907	22.2	21.3	51.3
Do	do	1907	22.2	20.5	51.3
Do	do	1908	14.8	28.4	58.1
Do	do	1908	14.7	28.8	58.2
California	Kubanka	1907	9.1	39.1	61.5
Do	do	1907	9.9	41.4	62.0
Do	do	1908	15.2	36.3	59.4
Do	do	1908	14.7	44.8	61.2
South Dakota	do	1907	13.9	37.9	63.0
Do	do	1907	12.9	39.4	63.7
Do	do	1908	16.6	24.9	57.8
Do	do	1908	16.5	29.8	60.2

TABLE V.—*Percentage of protein and phosphoric acid in grain from plots to which chemicals were applied at three stages of growth*

Chemicals applied	Series A. Protein (N×5.7).			Series A. Phosphoric acid (P_2O_5).			Series B. Protein (N×5.7).			Series B. Phosphoric acid (P_2O_5).		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	14.1	14.7	15.3	1.04	1.05	1.18	15.9	16.6	14.8	0.84	0.99	1.03
Potassium chlorid	13.2	13.1	12.6	1.15	1.24	1.02	13.6	13.2	13.3	1.16	1.14	1.15
Calcium nitrate		14.4	14.2	1.00	1.10	1.15	14.5	15.9	14.3	.92	1.03	1.07
Ammonium sulphate	13.5	14.5	12.8	1.00	1.05	1.14	14.4	15.6	14.0	.81	.98	1.07
Sodium hydroxid	12.3	12.4	12.8	1.13	1.05	1.18	13.4	13.3	13.1	1.17	1.10	1.14
Potassium nitrate	13.2	14.7	14.5	1.02	.98	1.12	15.0	16.9	13.5	.87	1.11	1.13
Sulphuric acid	12.4	12.5	12.3	1.12	1.14	1.21	13.2	13.2	12.9	1.13	1.14	1.16
Ammonium chlorid	12.4	13.9	13.0	.98	1.04	1.16	14.0	14.4	13.7	1.05	1.07	1.12
Potassium hydroxid	12.4	12.7	13.2	1.20	1.18	1.18	13.3	13.3	13.0	1.14	1.19	1.13
Ammonium nitrate	13.8	13.2	14.8	1.15	1.09	1.15	13.8	16.6	14.0	.99	1.06	1.17
Water		12.2			1.17			13.2			1.13	
Control		12.5			1.18			13.3			1.19	
Sodium chlorid	12.9	13.0	12.5	1.14	1.20	1.20	13.3	13.3	13.3	1.16	1.13	1.15
Calcium chlorid	12.5	12.7	12.9	1.16	1.19	1.18	13.4	13.7	13.2	1.14	1.16	1.17
Hydrochloric acid	13.0	12.8	12.5	1.18	1.18	1.15	13.5	13.3	13.2	1.15	1.13	1.20
Potassium sulphate	12.5	12.4	13.0	1.15	1.19	1.21	13.8	13.0	13.5	1.15	1.09	1.16
Magnesium nitrate	13.0	14.2	14.1	1.04	1.01	1.17	13.3	14.4	13.9	1.05	.99	1.08
Magnesium chlorid	12.9	13.2	12.9	1.15	1.18	1.14	13.8	13.0	13.2	1.08	1.12	1.11
Nitric acid	13.7	15.4	14.6	.98	.98	1.06	15.2	16.7	14.4	.94	.97	1.10
Ammonia		15.9	13.7		1.20	1.20		16.3	13.3		1.07	1.06
Water		12.5			1.11			12.9			1.11	
Control		12.6			1.10			13.6			1.12	

TABLE VI.—*Percentage of protein and phosphoric acid and straw from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Protein (N×6.25).			Phosphoric acid (P_2O_5).			Protein (N×6.25).			Phosphoric acid (P_2O_5).		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	3.27	3.45	2.84	0.18	0.17	0.19	3.98	4.06	3.74	0.12	0.15	0.17
Potassium chlorid	2.51	2.84	2.49	.23	.24	.19	3.45	3.26	3.04	.24	.25	.24
Calcium nitrate	2.91	3.78	3.09	.15	.20	.25	3.99	4.41	3.32	.16	.20	.23
Ammonium sulphate	3.58	3.91	3.26	.....	.....	.....	3.12	3.59	3.73	.12	.15	.24
Sodium hydroxid	3.54	2.92	3.35	.29	.26	.27	3.12	2.77	2.77	.24	.21	.20
Potassium nitrate	3.06	4.20	3.51	.20	.21	.21	3.90	4.07	3.05	.28	.19	.20
Sulphuric acid	2.72	3.06	3.21	.23	.26	.32	3.34	2.64	3.05	.25	.19	.19
Ammonium chlorid	3.39	3.61	2.93	.20	.20	.23	3.60	3.59	3.26	.18	.18	.20
Potassium hydroxid	2.72	2.51	2.66	.23	.22	.23	2.97	3.25	3.25	.22	.22	.21
Ammonium nitrate	2.92	3.65	3.99	.17	.19	.19	3.53	3.43	3.33	.16	.20	.21
Water		2.59			.19			2.51			.14	
Control		2.71			.21			2.62			.22	
Sodium chlorid	2.42	2.62	2.68	.21	.20	.21	2.68	2.85	2.86	.23	.21	.22
Calcium chlorid	2.49	2.01	2.48	.19	.21	.21	3.04	2.76	2.63	.24	.18	.22
Hydrochloric acid	2.89	2.49	2.75	.21	.21	.19	2.97	2.86	3.04	.23	.18	.22
Potassium sulphate	2.63	2.83	2.49	.20	.25	.26	2.63	2.64	2.86	.16	.19	.23
Magnesium nitrate	2.83	3.31	2.81	.16	.17	.18	3.12	3.45	2.84	.19	.16	.17
Magnesium chlorid	2.48	2.47	2.55	.21	.22	.20	2.65	3.03	3.32	.16	.20	.24
Nitric acid	3.02	4.55	3.36	.15	.20	.21	3.09	3.47	3.25	.17	.20	.21
Ammonia		3.99	3.03	.....	.20	.22	.....	.....	.....	.....	.18	.23
Water		2.82			.24		.....	.....	.....		.23	
Control		2.83			.22		.....	.....	.....		.25	

TABLE VII.—*Lack of correlation between nitrogen and phosphoric acid content of wheat grown in Arlington on plots to which various chemicals were applied*

Chemicals applied.	Stage of growth.	Protein (N×5.7).	Phosphoric acid (P_2O_5).	Chemicals applied.	Stage of growth.	Protein (N×5.7).	Phosphoric acid (P_2O_5).
		Per cent.	*Per cent.*			*Per cent.*	*Per cent.*
Sodium nitrate	2	16.4	1.02	Magnesium sulphate	3	11.3	0.96
Potassium nitrate	2	17.0	1.01	Potassium chlorid	2	11.45	1.03
Ammonium chlorid	1	17.1	1.00	Do	3	10.9	1.02
Ammonium nitrate	1	16.4	1.03	Calcium chlorid	3	11.7	1.01
Do	2	16.9	1.02	Potassium sulphate	1	11.2	1.03

TABLE VIII.—*Percentage of ash and silica in straw from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Ash.			Silica.			Ash.			Silica.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	11.0	11.3	13.0	8.1	8.0	10.8	11.2	11.9	12.2	6.1	7.3	8.0
Potassium chlorid	13.1	12.0	11.7	10.7	9.0	9.3	12.2	13.0	11.9	8.8	8.6	8.6
Calcium nitrate	10.0	11.8	12.8	5.8	7.3	9.0	11.0	11.1	11.7	6.7	6.5	8.4
Ammonium sulphate	9.42	9.60	11.3	5.5	5.6	8.0	11.1	11.2	11.5	7.2	7.0	8.3
Sodium hydroxid	12.5	12.8	13.2	8.9	9.6	10.2	14.3	12.5	12.8	10.5	8.9	9.3
Potassium nitrate	11.1	11.8	11.8	8.1	7.9	8.3	11.9	12.3	11.9	8.1	7.2	8.5
Sulphuric acid	13.0	13.7	13.6	9.9	10.6	10.6	12.2	13.3	11.2	9.0	10.0	7.5
Ammonium chlorid	11.1	11.1	12.8	7.9	7.8	9.9	11.0	10.8	12.2	7.5	7.9	9.2
Potassium hydroxid	14.0	12.1	12.3	11.0	9.2	9.2	11.8	11.1	10.8	8.6	8.3	7.9
Ammonium nitrate	10.7	12.6	11.6	7.3	9.3	8	10.6	10.7	13.0	7.3	7.2	9.7
Water		12.7			9.7			11.4			8.0	
Control		12.1			9.0			12.0			8.7	
Sodium chlorid	12.3	11.4	12.6	9.1	8.6	9.3	12.0	13.7	11.9	9.2	10.3	8.2
Calcium chlorid	12.5	12.1	12.8	9.4	9.2	10.4	12.9	12.0	12.4	9.7	9.0	10.7
Hydrochloric acid	12.1	11.6	11.6	9.2	8.9	8.8	12.2	12.9	14.3	9.0	9.3	10.9
Potassium sulphate	12.3	12.3	13.4	9.3	9.2	10.3	13.3	12.8	13.5	9.2	9.8	10.1
Magnesium nitrate	10.0	11.7	12.1	7.6	8.5	9.0	11.9	12.8	13.2	8.8	9.7	9.6
Magnesium chlorid	12.8	12.3	13.2	9.8	9.2	10.1	12.0	13.2	12.3	9.0	10.0	9.8
Nitric acid	10.3	10.9	10.8	7.0	7.4	7.5	10.3	11.2	11.6	6.8	7.9	8.2
Ammonia	.....	13.1	12.4	.....	9.7	8.9	.....	11.8	11.8	.....	7.8	8.3
Water		11.9			8.9			12.0			8.7	
Control		12.1			9.1			12.1			9.1	

RESULTS OF EXPERIMENTAL WORK

TOTAL YIELD AND PERCENTAGE OF GRAIN

The total yield represents the weights of the crops obtained immediately after they were harvested. The crops were harvested when fully ripe and after a continued dry period. The percentages of grain were determined in the samples, ranging from 1½ pounds to 2 pounds in weight, brought to Washington.

As seen from Table I, the plots which had received nitrogen in any form in the first stage gave distinctly higher yields, both as compared with the plots which had received no nitrogen and as compared with those which had received it in the other two stages. The application of the first stage was made later in the season than had been planned, and subsequent experiments have shown that the effectiveness of nitrogen with respect to increased yields diminishes as the season advances toward the completion of the vegetative stage.[5] On the other hand, as a result of the lack of uniformity in transition from stage to stage, at the time of the second-stage application part of the crops on each plot were still in the first stage. This probably accounts for the fact that the plots which had received their nitrogen in the second stage as a rule gave higher results than those which received it in the third stage.

The percentages of grain in the crops (Table I) were somewhat lower on the plots which had received nitrogen in the first stage than on the others. However, the lower range in the percentages of grain would not materially alter the grain relationship of the crops. The figures representing the total yields, therefore, are expressive of the relative yields of grain as well.

PERCENTAGE OF YELLOW-BERRY

The result of the lack of original uniformity in the plots, especially in those of series B, is most evident in the yellow-berry figures. Nevertheless, the fundamental principle that the application of nitrogen at the second stage is most effective in preventing yellow-berry was borne out very consistently. The percentage of yellow-berry is the lowest on the plots which received their nitrogen at the second stage. A marked decrease in yellow-berry is also shown by the crops which received their nitrogen in the first stage. This probably is due to the fact that the crops did not respond fully in yield because of a late application, and part of the nitrogen, applied at this stage and not utilized, remained in the soil during the second stage.

In some cases a somewhat decreased percentage of yellow-berry is shown by the crops which received their nitrogen in the third stage. This probably is due to the lack of uniformity in transition from one stage to another. At the time when most of the heads were in the milk stage, some of them had not yet reached this stage.

No conclusion can be drawn as to the effects of the direct and residual physiological acids and alkalies and their neutral salts, owing to the general lack of uniformity in the plots, which resulted in a too wide range of normal variation as indicated by control plots in both series, but especially in series B. Taking into consideration the fact that the

[5] DAVIDSON, Jehiel. THE EFFECT OF NITRATES APPLIED AT DIFFERENT STAGES OF GROWTH ON THE YIELD, COMPOSITION, AND QUALITY OF WHEAT. *In* Journ. Amer. Soc. Agron., v. 14, no. 4, p. 118–122. 1922.

effect of nitrogen in preventing yellow-berry was shown clearly in spite of all the disturbing factors, one would be justified in assuming that the effect of these chemical groups was inconsiderable, if any. This would seem to settle the practical question as to the possibility of preventing the characteristic coloration of the grain associated with yellow-berry by means other than nitrogen. However, the theoretical question as to the nature of the coloration of flinty grain and of that affected with yellow-berry remains unsettled. It is still possible that the coloration is an indicator reaction in response to physiological alkalinity and acidity. All the ammonium salts probably underwent nitrification before they were assimilated by the plants. Their effect, therefore, would be the same as that of nitrates. It is possible also that the brown color of the flinty kernels is caused by the action of the alkaline amino groups which might increase with the general increase in the nitrogen content on the hypothetical natural indicator present in the hull.

WEIGHT PER BUSHEL AND WEIGHT PER 1,000 KERNELS

No correlation can be observed between the different treatments, which resulted in different nitrogen contents, and the weight per bushel and the weight per 1,000 kernels (Table III). Taking the plots in groups of three for each of the nitrogen carriers, it would seem that those which had received their nitrogen in the first stage gave a somewhat lower weight per 1,000 kernels than those which had received it in the second and the third stages. The first stage, however, is not the one which gave the highest protein contents. These results, as well as those obtained in Kentucky,[6] fail to disclose any direct relation between the protein content and the weight per bushel and, especially, the weight per 1,000 kernels. Cases are on record, however, in which such a correlation has been reported. Table IV, compiled from data reported in Bulletin 128 of the Bureau of Chemistry, United States Department of Agriculture,[7] will serve as an illustration. This table shows a reverse correlation between the nitrogen content and the weight per 1,000 kernels and weight per bushel for Kansas and South Dakota, but no correlation for California, as was the case in the experiments here reported.

What is the explanation of these seemingly contradictory results? Within the limits of the bureau's experiments, it has been established that the presence of nitrogen at the time of heading is conducive to a high nitrogen content. The conditions of these experiments would suggest the distinction between a physical and physiological abundance of available nitrogen in the soil. The term "physiological abundance" is used to signify a condition in which the crop, being depressed in its development, is kept from drawing upon the store of available nitrogen in the soil to the full normal extent. Any environmental conditions which depress the normal development of the crop cause a physiological abundance of available nitrogen. Under such circumstances, the factors which are responsible for the abundance of available nitrogen are responsible also for the subnormal development of the grain. Hence, the coincidence of a high protein content and a low weight per 1,000 kernels and a low weight per bushel. In these experiments, the abundance of

[6] DAVIDSON, J., and LECLERC, J. A. OP. CIT.

[7] LECLERC, J. A. TRI-LOCAL EXPERIMENTS ON THE INFLUENCE OF ENVIRONMENT ON THE COMPOSITION OF WHEAT. U. S. Dept. Agr. Bur. Chem. Bul. 128, 18 p. 1910.

available nitrogen at the time of heading was distinctly physical, which may be the reason why no such correlation was found. It would seem, therefore, that there is no direct correlation between the protein content and the weight per 1,000 kernels and the weight per bushel of wheat.

PROTEIN CONTENT [8]

The results reported in Table V show that the application of nitrogen at the second stage gave the highest nitrogen contents. Although series A proved to be somewhat irregular, of the nine nitrogen carriers used six were consistent with the general tendency. Series B was wholly consistent. The irregularities, as well as the fact that increased nitrogen contents were obtained from the first and third application, can be explained in the same way as the irregularities with reference to yellow-berry. The increased nitrogen content, which resulted from the first stage application, was due to the fact that not all the nitrogen was used up during the first stage. The increased nitrogen content resulting from the third stage application can be explained by the lack of uniformity in transition from one stage to another, already discussed. The same causes are responsible for the fact that the increases in nitrogen obtained from second stage applications are not as sharp as they were in the bureau's previous experiment.[9] While the first application showed an increase in nitrogen, due to the fact that not all the applied nitrogen had been utilized during the first stage, the nitrogen content from the plots which received their nitrogen at the second stage did not reach its proper height, for the reason that part of the nitrogen of this application was utilized in making vegetative growth as was shown by the increased yields from these plots.

The nitrogen content of the straw (Table VI) shows the same tendencies as that of the grain.

PHOSPHORIC-ACID CONTENT

Headden[10] found that the application of sodium nitrate to wheat plots caused a depression in the phosphoric-acid content of the grain. In the bureau's previous experiment,[11] where sodium nitrate had been applied on a number of plots at the three stages of growth, no correlation between the application of nitrogen and the phosphoric-acid content was disclosed. In the present experiment, however, a correlation is observed between the application of nitrogen and the phosphoric-acid content, both in the grain and in the straw. As seen from Table V, the phosphoric-acid content in the grain from the plots which received their nitrogen in the first and second stages is consistently lower than that in the grain from the plots which received it at the third stage and the controls which, in this case, may include all the plots which received chemicals other than nitrogen carriers. The only exceptions are the

[8] It is understood that the term "protein" represents values obtained by multiplying the nitrogen content, determined in the regular way, by certain factors. These factors vary with the substance analyzed. In this paper the factors used were 5.7 for the grain and 6.25 for the straw. It has been the general practice in agricultural literature to express the nitrogen values in terms of protein. This practice has been established because of the fact that the main bulk of the nitrogen in mature plant substance is protein in character, and because of the accepted food value associated with proteins.

[9] DAVIDSON, J., and LECLERC, J. A. OP. CIT.

[10] HEADDEN, W. P. A STUDY OF COLORADO WHEAT, PART III. Colo. Agr. Exp. Sta. Bul. 219, 131 p. 1916.

[11] DAVIDSON, J., and LECLERC, J. A. OP. CIT.

ammonium-nitrate plots in series A and the ammonia plots in both series. There would seem to be a tendency toward a greater depression in the plots of the first application. This tendency shows itself more clearly in series B.

On the other hand, results obtained on the Arlington Farm, in an experiment carried out on lines similar to these, again failed to disclose any correlation between the application of nitrogen and the phosphoric-acid content (Table VII).

It would seem, therefore, that there are conditions under which the application of nitrogen causes a depression in the phosphoric-acid content of the crop and that under other conditions the application of this element has no effect on the phosphoric acid content. What factors are involved in these seemingly contradictory tendencies?

Of the four cases cited here it happens that the two (Colorado and Nebraska), which show a correlation between the application of nitrogen and the phosphoric-acid content, deal with hard winter wheats and the other two (Kentucky and Arlington Farm), which show no such correlation, deal with soft winter wheats. This would suggest the possibility that these two groups react differently toward an abundance of available nitrogen. The underlying principle then would be, in the case of the hard winter wheats, some interchange between phosphorus and nitrogen, based on the similarity of their functions. The depressed phosphoric-acid content would be directly correlated with the high protein content. This assumption, however, does not seem to be borne out by the results in Table V. In those cases where the third application of nitrogen resulted in a high protein content, especially in series A, no depression in the phosphoric-acid content can be observed.

Another possible factor accounting for the different effects of applied nitrogen on the phosphoric-acid content is the supply of available phosphoric acid in the soil. It may be assumed, then, that when the supply of available phosphorus is limited the stimulation of growth, caused by the application of nitrogen, creates a physiological scarcity of this element. This assumption would seem to be borne out, in a general way, by the results here obtained. The application of nitrogen at the first two stages which resulted in increased yields also caused a depression in the phosphoric-acid content. There is also an apparent tendency toward a greater depression resulting from the first application, which produced the highest yields. This assumption, however, would not seem to be borne out by Headden's results. In his experiments the application of sodium nitrate did not cause an appreciable increase in yield, if any. Nevertheless, it produced consistent depressions in the phosphoric-acid content.

It is clear that under certain conditions the presence of nitrogen interferes with the assimilation of phosphoric acid by the plant. It is not unlikely that this interference is indirect. Under certain conditions the nitrogen may be instrumental, through its action on the soil flora, in transforming the available phosphoric acid into less available forms.

The phosphoric acid in the straw follows the same tendency as that in the grain (Table VI).

The ash and potash contents of the grain were also determined, but as the results failed to disclose any consistent tendencies they are not given.

ASH AND SILICA CONTENT IN THE STRAW

As seen from Table VIII, the application of nitrogen at the first and second stages caused a distinct depression in the ash and silica content of the straw.

A depression in the silica content of the straw, caused by the application of sodium nitrate, was also observed by Headden.[12] In his case the depression was slight. In the bureau's experiments the difference between the first two stages and the third stage, for each nitrogen carrier, was always more than 1 per cent. The third stage plots, not being affected by the application of nitrogen with reference to the silica content of the straw, may be considered as controls. The sharper differences are due probably to the exceptionally high silica content of the straw in this experiment. Ammonia is the exception. It did not depress the silica content in either series. Attention is called to the fact that the same chemical also failed to produce a depression in the phosphoric-acid content of the grain. The relationship between the phosphoric-acid depression and the depression of silica in the straw is further accentuated by the fact that ammonium nitrate, which failed to depress the phosphoric acid in the grain from the second stage plot in series A, also failed to depress the silica content of the straw from the same plot. No explanation of the theoretical principles involved in depression of silica can be offered at present.

As to the bearing of this depressed silica content in the straw on lodging, frequently caused by the application of nitrogen, it is hard to draw conclusions from the present experiment. No lodging was observed on any of the experimental plots. It is possible that as the silica content of the straw in this experiment was exceptionally high, even the depressed silica content was sufficient to sustain the strength of the stalk. It is probable also that the depressed silica content is only one of the factors which cause lodging, or it may be no factor but only coincident with the real factors resulting from the application of nitrogen, which, under certain conditions, cause lodging of the crop.

SUMMARY

(1) The application of nitrogen in any of the inorganic forms used in the early stages of growth was instrumental in producing the highest yields of wheat.

(2) The application of nitrogen in any of the inorganic forms used at the time of heading was instrumental in producing the best quality of grain with reference to "yellow-berry" and the protein content. It also produced a high protein content in the straw.

(3) No relation between the nitrogen content and the weight per 1,000 kernels and weight per bushel of wheat was disclosed in this experiment. The apparent disagreement between these results and the results of other experiments, which establish a relation between these two factors, is explained by the distinction between a physical abundance and a physiological abundance of nitrogen created by the failure of the crop to develop normally.

(4) No differences could be observed in the effect of the different forms of inorganic nitrogen.

[12] Headden, W. P. op. cit.

(5) No effect was produced by the chemicals, other than the nitrogen carriers, on the yield of the crop or on the quality of the grain.

(6) The application of nitrogen at the first two stages caused a depression of the phosphoric-acid content in the grain, as well as in the straw. In the light of these results, this depression could not be considered either as a yield relation nor as a reciprocal relation of the two elements in the plant tissues, based on similarity of function.

(7) The application of nitrogen, at the first two stages of growth, caused a marked depression of the ash and silica content in the straw.

POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN)[1]

By O. F. BLACK, *Chemical Biologist*, W. W. EGGLESTON, *Assistant Botanist*, and J. W. KELLY, *Chemical Laboratorian, Office of Drug, Poisonous, and Oil Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and H. C. TURNER, *Assistant Animal Husbandman, Virginia Agricultural Experiment Station*

INTRODUCTION

Since the time of the early settlements in the mountains of Virginia frequent fatal cases of poisoning have occurred among cattle grazing in the mountain pastures in early spring. It has long been believed that certain early spring plants popularly known as "staggerweeds" have been the cause of these fatalities, since these plants are among the first to appear in the pastures and are often eaten by cattle when other forage is not abundant. Suspicion has chiefly centered upon the plants commonly called larkspur (*Delphinium tricorne* Michx.), dutchman's-breeches (*Bikukulla cucullaria* (L.) Millsp.), squirrel-corn (*B. canadensis* (Goldie) Millsp.), and wild bleeding heart (*B. eximia* (Ker) Millsp.). In the literature relating to poisonous plants the toxic character of *D. tricorne* has long been recognized, but the American species of Bikukulla appear to have received comparatively little attention from chemists and practically none from toxicologists.

The probable poisonous character of species of Bikukulla was first brought to the attention of the Department of Agriculture in June, 1920, by Prof. H. S. Stahl, of the Virginia Polytechnic Institute. He submitted specimens of "little staggerweed," later identified as *Bikukulla cucullaria*, with the statement that this plant was believed to be responsible for the recent death of a number of cattle in the mountain pastures of Bland County, Va. An extract of the plant prepared in the laboratory was found to be highly toxic, and in order to secure material for further study a representative of the department visited the locality where the cases of poisoning had occurred. At the request of Dr. A. W. Drinkard, jr., Director of the Virginia Agricultural Experiment Station, arrangements were made to conduct some cooperative experiments with a view to determine by feeding tests the effect upon cattle of the plant material collected for chemical examination.

As a result of these investigations *Bikukulla cucullaria* has been shown to contain a poisonous alkaloid heretofore unrecognized, and the toxicity of this plant for cattle has been demonstrated. Poisonous alkaloids have also been found in *B. canadensis*, but this species is much less toxic than *B. cucullaria* and apparently is not likely to cause any damage to cattle.

[1] Accepted for publication Sept. 13, 1921. The plants referred to in the title are also known in botanical literature under the following genus names: Dicentra, Bicuculla, Dielytra, Diclytra, Corydalis, Fumaria, and others.

Journal of Agricultural Research, Washington, D. C.

Vol. XXIII, No. 2
Jan. 15, 1923.
Key No. G-262.

SYSTEMATIC POSITION OF "LITTLE STAGGERWEEDS"

BIKUKULLA

Perennial and smooth herbs with basal ternately compound, dissected delicate leaves; watery juice; horizontal rootstocks and scapose, racemose, nodding inflorescence. Flowers flattened, either deciduous or withering persistent. Pedicels 2-bracted. Sepals 2, scalelike. Petals 4, the two outer spurred at base, loosely united; the two inner pair narrower, and their callous-crested tips united over the stigmas. Stamens 6, in two groups opposite the outer petals, hypogynous; their filaments often united; middle anthers of each set 2-celled, the lateral ones 1-celled. Pod 10 to 20 seeds, seeds crested.

A genus of about 13 species, ranging across North America to Eastern Asia. Six species are known on the Pacific slope of America and 3 on the Atlantic watershed.

The bleeding heart (*Bikukulla spectabilis* (L.) Coville) of the gardens is an Asiatic species.

KEY TO ATLANTIC SPECIES

Racemes simple; rootstocks tuber-bearing.
- Rootstocks long, with scattered cornlike, yellow tubers; flowers cordate; spurs rounded; inner petals conspicuously crested..... 1. *B. canadensis.*
- Rootstocks much shortened; tubers gathered in a scaly, granulated bulb, white, becoming pink and dark red; flowers sagittate; spurs spreading, elongated; inner petals minutely crested..... 2. *B. cucullaria.*

Racemes compound; rootstocks scaly, not tuber-bearing..... 3. *B. eximia.*

1. *Bikukulla canadensis* (Goldie) Millsp. Squirrel-corn. Turkey-corn. Turkey-pea. Little blue stagger. Trembling stagger. Staggerweed. Colicweed. Wild hyacinth.

Foliage bright green; scapes erect 6 to 12 inches high, overtopping the leaves; flowers greenish white, tinged with pink. On vegetable mold in woods. April and May. Nova Scotia, Ontario, Minnesota, Missouri, and south along the mountains to North Carolina and Tennessee. In Virginia it occurs abundantly on northern slopes in the Alleghany Mountains and often in the Blue Ridge.

It rises to 4,500 feet altitude on White Top Mountain, although it seems most at home in the neighborhood of 3,000 feet. Gorge of the Potomac above Washington. Rare.

2. *Bikukulla cucullaria* (L.) Millsp. Dutchman's-breeches. Little blue stagger. Trembling stagger. Staggerweed. Colicweed. Soldier's-cap. Whitehearts. Indian boy-and-girls. Butterfly banners, etc.

Foliage often glaucescent; scapes 6 to 12 inches high, usually overtopped by the leaves; flowers white with yellowish tips. April and May.

On vegetable mold in the woods. Growing in Virginia with *Bikukulla canadensis* and *Delphinium tricorne* but usually preferring better drained soil than the former. Nova Scotia, Ontario, Minnesota, Nebraska, Kansas, and Missouri, and south in the mountains to Alabama. Common on the northern slopes of the Alleghany Mountains and often in the Blue Ridge, being more abundant around 3,000 feet altitude and rising on White Top Mountain to about 4,500 feet. Gorge of the Potomac above Washington.

3. *Bikukulla eximia* (Ker) Millsp. Wild bleeding heart. Staggerweed. Turkey-corn.

Leaves dark green; scapes 1 to 2 feet high; longer than the leaves; flowers deep pink. Mountain rocks and river gorges, southern Appalachian Mountains from Wills Mountain, Allegany County, Md., south to North Carolina and Tennessee.

CHEMICAL EXAMINATION

As might be expected in a group so nearly related to the poppy family, species of Bikukulla have been found to contain alkaloids. Wenzell (7)[1] in a study of *Bikukulla canadensis* (*Corydalis formosa*) reports the presence of an alkaloid which he assumed, on very insufficient evidence, to be corydaline. Fischer and Soell (3) made an examination of *B. cucullaria* (*Dicentra cucullaria*) and were able to identify protopine and also to isolate minute quantities of two other alkaloids in crystalline form, which they characterized only by their melting points and certain other physical properties. Battandier (2) discovered protopine in *B. formosa* (DC.) Howell (*D. formosa*). Heyl (5) made a more thorough study of the same plant and observed that protopine was the chief alkaloid, besides isolating two others which he differentiated by their physical properties.

Gadamer (4) found protopine in *B. spectabilis* (*D. spectabilis*), with a yield of 1 per cent, and surmised the presence of other alkaloids. Asahina (1) obtained a crystalline alkaloid from *B. pusilla* (Sieb. & Zucc.) Coville (*D. pusilla*), which he named dicentrine, and also isolated protopine from the plant. He made a very complete chemical study of dicentrine and concluded that it was identical with one of the unnamed alkaloids found by Heyl in *B. formosa* having a melting point of 169° C. Dicentrine was studied from a pharmacological standpoint by Iwakawa (6), who found that it produced narcosis when administered to small animals in moderate doses, whereas larger quantities caused convulsions, weakening of the heart, and respiratory paralysis.

A search of the literature has failed to reveal any publication on the constituents of *Bikukulla eximia*.

Table I brings out the more essential facts in the preceding summary.

TABLE I.—*Comparative chemical analyses of species of Bikukulla made by various investigators*

	BIKUKULLA CUCULLARIA, Fischer and Soell.		
Alkaloids	Protopine.		
Melting point	206° C.	231° C.	215° C.
Solubility	Soluble in alcohol.	Insoluble in alcohol.	Soluble in alcohol.
	Soluble in ether.	Soluble in $CHCl_3$.	
Form	Cryst. pr.	Needles, rosettes	Fine granular.
Color tests:			
Concentrated H_2SO_4		Red to Brown.	
Erdmann's		Red to violet.	
Froede's		Red to violet.	
Concentrated HNO.		Red to yellow.	

[1] Reference is made by number (italic) to "Literature cited," p. 78.

TABLE I.—*Comparative chemical analyses of species of Bikukulla made by various in vestigators*—Continued.

BIKUKULLA FORMOSA, G. Heyl.

Alkaloids	Protopine		
Melting point	201° to 202° C.	168.5° C.	142.5° C.
Solubility		Soluble in H Br. salt.	Soluble in H Br salt.
		Insoluble in alcohol.	Soluble in alcohol.
Form	Needles	Yellow needles	White needles.
Color tests:			
Concentrated H_2SO_4.	Yellow	Colorless to red violet.	Colorless.
Erdmann's	Yellow to green	Blue	Weak green.
Froede's	Violet	Deep blue	Blue green.
Concentrated HNO.	Colorless	Colorless to brown	Brown.

BIKUKULLA PUSILLA, Y. Asahina.

Alkaloids	Protopine	Dicentrine.
Melting point	207° C.	168–9° C.
Solubility	Soluble in alcohol	Soluble in hot alcohol.
	Soluble in ether	Soluble in $CHCl_3$
Form	Prisms	Prisms.
Color tests:		
Concentrated H_2SO_4	Yellow to blue	Colorless to violet.
Erdmann's	Yellow to violet	Blue.
Froede's	Violet to blue	Deep blue.
Concentrated HNO	Colorless to brown	Colorless to brown.

It will be seen from Table I that protopine seems to be the alkaloid most common to the members of the genus, and if Asahina's (*1*) supposition is correct dicentrine is common to *Bikukulla formosa* and *B. pusilla* and possibly may be contained in others of the group. It seems not improbable that the protopine of Heyl (*5*) and Gadamer(*4*), melting at 201° C., may be a different body from that of Fischer(*3*) and Asahina(*1*), melting at 207° C. Some of the work summarized above is very incomplete, with conclusions often based on color reactions, which are notoriously deceptive. The physiological action of these alkaloids had not been studied, with the exception of protopine, which has long been known as one of the opium group, and dicentrine in Iwakawa's(*6*) report. Neither of these two alkaloids would seem to possess sufficiently toxic qualities to account for the symptoms of poisoning in animals fed with *B. cucullaria* (Pl. 1, A) and *B. canadensis* (Pl. 1, B).

The material on which the following chemical work was done was gathered at Round Bottom, Va., in April, 1921, just previous to the flowering stage. The tops and roots of *Bikukulla cucullaria*, and only the roots of *B. canadensis* were used in the experiments. The tops of the *B. cucullaria* were dried and in good condition, but the roots of both plants were contaminated with soil which was removed by sieving in a stream of running water. They were then dried, first in the air and then in an oven at 100° C., and ground fine in a mill.

The object of the study here reported was to determine what constituents of the plants were responsible for their toxic effect, and as it was known that both contained alkaloids it was obviously necessary to isolate and test the toxicity of these compounds. As a preliminary experiment a few grams of the ground roots of the two plants were allowed to macerate in Prolius' solution for 48 hours. The solution was then decanted and evaporated spontaneously until nearly dry, and the residue was extracted with dilute hydrochloric acid. Both these solutions gave a heavy precipitate when tests were made with Mayer's reagent, showing the presence of alkaloids. The acid solutions were then made strongly ammoniacal, which threw down some of the dissolved material as a white flocculent precipitate, and were shaken out exhaustively with ether. After the ether evaporated, the residue was again taken up with very dilute hydrochloric acid. One-half cc. of each of these two solutions, which represented the raw alkaloids of *Bikukulla cucullaria* and *B. canadensis*, was injected subcutaneously into two white mice. The animal receiving the dose of *B. canadensis* gave some slight evidence of narcosis but suffered no other ill effects. The mouse injected with the *B. cucullaria* extract also behaved in a sleepy manner for some time and died unobserved inside of two hours. Another mouse was then given a similar injection of the same *B. cucullaria* extract, and his reactions were carefully followed. The animal showed no striking symptoms in the course of an hour, then suddenly grew restless for a few minutes, was seized with acute convulsions, and died almost immediately. These results seemed to indicate that the roots of *B. cucullaria* were poisonous, whereas those of *B. canadensis* were harmless, but upon further investigation this conclusion was modified.

An approximate assay of the total alkaloids in the material which was available, namely, the tops and roots of *Bikukulla cucullaria* and the roots of *B. canadensis* was undertaken as follows: Of each of these three samples 5 gm., dried and finely ground, were macerated for 48 hours in 50 cc. of Prolius' solution, the solution was decanted off and the residue washed with a little alcohol and added to the solution, which was then extracted with 50 cc. of 5 per cent H_2SO_4 in three portions. The acid extract was made alkaline with ammonia and shaken out exhaustively with ether. The ether solution was distilled to small bulk, transferred to a tared beaker, and allowed to evaporate spontaneously, whereupon it was completely dried in a desiccator and weighed. Table II shows the results of the three determinations.

TABLE II.—*Assay of total alkaloids found in Bikukulla cucullaria and B. canadensis*

Plant.	Weight of plant.	Alkaloid.		Character of residue.
			Per cent.	
B. cucullaria:	*Gm.*	*Gm.*		
Tops	5	0.062	1.24	Amorphous.
Roots	5	.080	1.60	Amorphous, with some needles.
B. canadensis:				
Roots	5	.157	3.14	Amorphous, with rosettes.

With known weights of the raw alkaloids from definite quantities of the three samples, the way was now open for comparative tests of their toxicity. To this end all three total alkaloidal residues were dissolved in exactly 5 cc. of very dilute acetic acid, and 0.5 cc. of each was injected subcutaneously into three mice. The result was the same in each instance the animal died almost instantly in violent convulsions. The dosage was as follows:

B. cucullaria:
Tops.....0.5 gm. plant=0.0062 gm. alkaloid.
Roots.....0.5 gm. plant= .0080 gm. alkaloid.
B. canadensis:
Roots.....0.5 gm. plant= .0157 gm. alkaloid.

By testing these solutions at gradually increasing dilutions it was possible to determine within narrow limits the minimal fatal dose of the three with respect to mice of about 20-gm. weight, as shown in Table III.

TABLE III.—*Minimal fatal dose of Bikukulla cucullaria and B. canadensis for mice of 20-gm. weight*

Plant.	Weight of plant.	Alkaloid.	Total dose per kilogram of body weight.
B. cucullaria:	*Gm.*	*Gm.*	*Gm.*
Tops	0.040	0.0005	0.02
Roots	.031	.0005	.02
B. canadensis:			
Roots	.250	.0100	.40

From these figures it can be estimated that the combined alkaloids of *Bikukulla cucullaria* roots and those of the tops are of approproximately the same degree of toxicity.

This finding runs counter to observations made in the field tests on calves, as reported elsewhere, in which 60 pounds of the tops were fed without effect. A possible explanation of this result is that the bulk and succulence of the fresh tops so dilutes the poison that its action is minimized and it is absorbed so gradually that the animal suffers no ill effects. Nevertheless the fact remains that the tops contain the poison and must be held partially accountable for the poisoning of grazing stock which eat the whole plant. Applying the foregoing figures to a comparison of the relative toxicity of the roots of *Bikukulla cucullaria* and *B. canadensis*, it will be seen that the alkaloids of *B. cucullaria* are about 20 times as poisonous as those of *B. canadensis*, but as there is a considerably larger percentage of alkaloids in *B. canadensis* the ratio of toxicity between the two plants would be reduced to approproximately 6 to 1.

As a preliminary experiment to acquire some information about the character of the alkaloids in the two plants under investigation, 100 gm. of each were extracted in a Sohxlet apparatus with the selective solvents, ether, chloroform, alcohol, and acidified water. In the case of *Bikukulla cucullaria* the acidified water gave only the faintest of tests with Mayer's reagent, showing that the other three solvents had made a complete extraction. All three solutions contained appreciable quantities of alkaloids. From the chloroform and alcohol fractions only amorphous

products were obtained, but from the ether extract a crystalline alkaloid was isolated. After the ether was distilled off the residue was triturated with dilute hydrochloric acid until all the alkaloid was removed and the acid solution was made ammoniacal and shaken out with ether. The etherial solution was concentrated to small bulk, the same volume of alcohol was added, and on slow evaporation there were deposited warty masses of colorless crystals. These were removed and pressed between filters and recrystallized from a mixture of alcohol and chloroform. After one recrystallization they melted at 168° C., which was not changed by a further purification of the same kind. It was at once assumed that the dicentrine of Asahina (*1*) had been isolated, but after testing the compound on mice it was evident that it was another substance, and one of much higher degree of toxicity. By methods already described, it was found that 0.04 mgm. of the alkaloid was fatal to a mouse. The animal exhibited a period of intense excitement followed by convulsions and death, whereas Iwakawa in trying out dicentrine on mice found the fatal dose to lie between 5 and 10 mgm., and his animals experienced a prolonged period of narcosis before death. These facts would seem sufficient to differentiate the two compounds.

Another alkaloid is known with a melting point of 169° C., namely, Y-homochelidonine, found chiefly in *Sanguinaria canadensis* L. It has always been prepared in conjunction with its physical isomeride B-homochelidonine, melting point 159°, but no trace of the latter was found in our product. Moreover, the physiological action of Y-homochelidonine is like morphine, chiefly narcotic. It is therefore reasonable to assume that the alkaloid isolated from *Bikukulla cucullaria* is new, and we propose to name it provisionally cucullarine. Unfortunately, the small quantity at our disposal has made it impossible to make a complete study of it, but this we intend to do later when larger quantities of material are available. We have been able, however, to note the following properties: It crystallizes in characteristic warty masses of prisms; it is colorless when first prepared but changes to pink in the light; it is soluble in ether and chloroform, but not so soluble in alcohol; it is precipitated by the usual alkaloid reagents, Mayer's, iodin in potassium iodid, picric acid (crystalline), and platinum chlorid; with concentrated sulphuric acid a small crystal dissolves with difficulty, producing a violet coloration turning brown.

Another portion of *Bikukulla cucullaria* was exhaustively extracted with 95 per cent alcohol containing a little acetic acid, the alcohol was removed by vacuum distillation, and the residue was taken up in dilute acetic acid and allowed to stand for some time. The small quantity of resin which separated was filtered off, and the solution was made alkaline with ammonia and shaken out first with ether then with chloroform. Very little alkaloid was recovered from the latter extract, the great bulk being removed by the ether. The etherial solution was set aside for several days with the expectation that protopine would crystallize out. This did not occur, however. The ether was then distilled off, and the raw alkaloid fractioned in various ways with the production only of amorphous products. Our failure to isolate protopine, reported by Fischer and Soell in *B. cucullaria*, may be due to the comparatively small quantities of material on which the work was done or to the stage of maturity of the plant. Fischer and Soell do not say when their *B. cucullaria* was gathered and are rather obscure in their description of the technic they employed.

As with *Bikukulla cucullaria* it has not been possible as yet to give the alkaloids of *B. canadensis* a complete survey. It seems highly probable that the latter plant contains cucullarine, as the physiological effects produced on mice by the raw alkaloids of both plants are so similar. If present in *B. canadensis* it undoubtedly must be there in very minute quantity, as the toxicity of *B. cucullaria* is so much greater. This may account for the failure so far to isolate it. However, a crystalline alkaloid from the roots of *B. canadensis* has been prepared. The material was percolated with 95 per cent alcohol, plus acetic acid, and the solution was treated in the usual way. By extracting this preparation with ether which contained a small quantity of alcohol a bright yellow compound was obtained, crystallizing in bundles of silky needles. It has a melting point of 210° C. and gave reactions with the customary alkaloid reagents and a crystalline precipitate with picric acid. It proved to be nontoxic when injected into a mouse. A dose of 0.6 mgm. caused the animal no apparent inconvenience.

It is proposed to make a further study of the alkaloids of *Bikukulla cucullaria*, *B. canadensis* and *B. eximia* in more detail with larger quantities of material.

FEEDING EXPERIMENTS

Feeding experiments with both *Bikukulla cucullaria* and *B. canadensis* were made by the representative of the Virginia Agricultural Experiment Station on a farm near Merriam, Bland County. The material used was collected on this farm and fed in a fresh condition. The animals used were healthy yearling steers, weighing approximately 275 pounds each.

At first these plants only were fed, but the animals ate of them so sparingly that it was found necessary to mix them with grass before they were taken freely. Account was kept of the weight of the grass and of the plants fed to each animal and of the portion which remained uneaten, from which the approximate weight of the suspected plants eaten was calculated. A detailed account of the experiments follows.

STEER 2.—The whole plant of *Bikukulla cucullaria* was given this animal. Feeding was begun at 2 p. m., April 19, 1921, but on that day only ½ pound of the plant was eaten; April 20, 3½ pounds were consumed between 7.30 a. m. and 5 p. m., without noticeable effects. Feeding was resumed at 5.15 p. m.; and at 5.30, when the animal had eaten about another pound of the plants, symptoms of poisoning were first exhibited. He suddenly began to tremble, ran backward then forward several times with the head held very high. He was frothing at the mouth and several times ejected partly digested stomach contents a distance of several feet. The trembling which occurred all over the body became more violent, convulsions ensued, and at 5.40, 10 minutes after the first seizure, he fell and was unable to rise. He lay upon the left side, with the head thrown back as in opisthotonos, and with the legs extended and rigid. He moaned as though in great pain, the eyes were glassy, breathing was very difficult, and the bowels were lax. About 5.50 relaxation began; the animal struggled but was unable to rise until 6.10, when he got up; and although very weak and nervous he was able to walk about. From this time his condition improved rapidly, and, when no other symptoms of poisoning appeared, he was returned to the pasture at noon, April 21.

STEER 5.—The bulbs alone of *Bikukulla cucullaria* were fed to this animal. Feeding was begun at 2 p. m., April 19, but practically none of

the material was eaten until the following day when approximately 5 pounds were consumed between 7.30 a. m. and 5.30 p. m. Then trembling, convulsions and all other symptoms manifested by steer 2 were exhibited. As in the case of steer 2 this aninmal was eating heartily of the bulbs at the time of seizure.

There was no appreciable difference in the time both animals were down or in their condition during recovery. These two cases agree so closely in every respect that they are regarded as practically identical.

STEER 4.—Between April 19 and April 25 this animal received 60 pounds of *Bikukulla cucullaria* tops which included the flowers. There were no ripe seeds in the material. The quantity consumed daily ranged from 4 to 11½ pounds. No symptoms of poisoning were observed during the seven days the animal was under observation.

STEER 3.—This animal consumed approximately 55 pounds of the entire plant of *Bikukulla canadensis* between 5.30 p. m., April 19 and 12.30 p. m., April 27. The quantity consumed daily ranged from 3½ to 9½ pounds. Aside from a slight restlessness and uneasiness on the second day of feeding, when 7½pounds of the plant were eaten, no injurious effect was observed.

STEER 6.—This animal was brought in for feeding on the afternoon of April 24, and was given the entire plant of *Bikukulla canadensis*. Feeding was continued until noon, April 27, up to which time he had consumed 16 pounds of this material. Six and one-half pounds of the plant were eaten on April 24, and on April 25 the animal was restless and uneasy practically all day, but no other symptoms were manifested.

These experiments confirm the popular opinion that *Bikukulla cucullaria* is poisonous to cattle, but more definite feeding experiments must be performed with *Bikukulla canadensis* before it can be positively stated that this species constitutes a menace to live stock.

CONDITIONS UNDER WHICH POISONING MAY OCCUR

Most of the cases of poisoning occur early in the spring. The danger is usually past by the middle of May, although in some of the higher altitudes poisoning may occur as late as June. Poisoning is said to occur most frequently following heavy rains, when, the soil being soft, the bulbs of the plant are mostly likely to be pulled up and eaten. The experiments show that both species of *Bikukulla* used are unpalatable to cattle and therefore unlikely to be eaten in harmful quantities when suitable forage is available.

SUMMARY

(1) *Bikukulla culcullaria* and *B. canadensis*, in Virginia popularly called "little staggerweeds," have long been considered poisonous to cattle.

(2) Chemical examination has shown that both these plants contain toxic alkaloids and that the tops as well as the bulbs of *B. cucullaria* are poisonous.

(3) *B. cucullaria* contains at least one alkaloid of a highly poisonous nature. This alkaloid, heretofore apparently unknown, has been named cucullarine, and its properties are described.

(4) Cucullarine probably occurs in *B. canadensis* also, since its physiological effect on mice closely resembles that of *B. cucullaria*.

(5) Feeding experiments show that *B. cucullaria* is toxic for cattle.

LITERATURE CITED

(1) ASAHINA, Y.
1909. UEBER DIE ALKALOIDE VON DICENTRA PUSILLA SIEBR. ET ZUCC. *In* Arch. Pharm. Bd. 247, Heft 3, p. 201–212.

(2) BATTANDIER, J. A.
1892. PRÉSENCE DE LA FUMARINE DANS UNE PAPAVÉRACÉE. *In* Compt. Rend. Acad. Sci. Paris, t. 114, no. 20, p. 1122–1123.

(3) FISCHER, R., and SOELL, O. A.
1902. THE ALKALOIDS OF DICENTRA CUCULLARIA. *In* Pharm. Arch., v. 5, no. 7, p. 121–124.

(4) GADAMER, J.
1901. UEBER DICLYTRA SPECTABILIS. *In* Apoth. Ztg., Jahrg. 16, No. 71, p. 621.

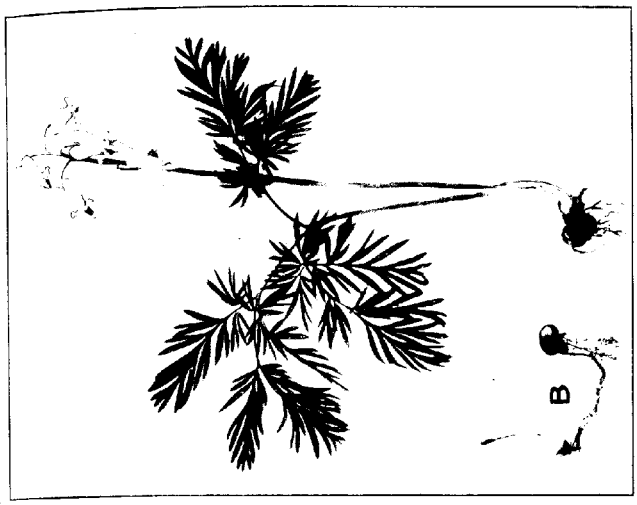
(5) HEYL, Georg.
1903. UEBER DIE ALKALOIDE VON DICENTRA FORMOSA (ANDR.) DC. *In* Arch. Pharm., Bd. 241, Heft 4, p. 313–320.

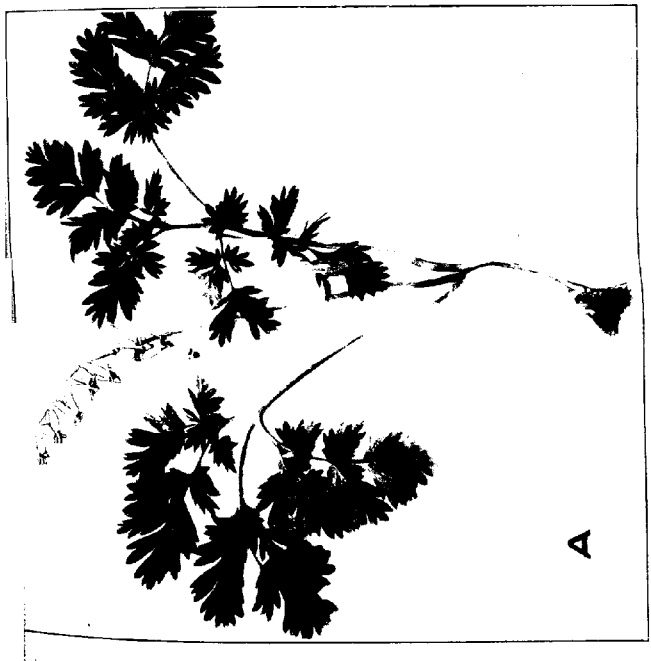
(6) IWAKAWA, K.
1911. PHARMAKOLOGISCHE UNTERSUCHUNGEN ÜBER "DICENTRIN", DAS ALKALOID DER DICENTRA PUSILLA SIEB. ET ZUCC. *In* Arch. Exp. Path. u. Pharmakol., Bd. 64, Heft 5/6, p. 369–382.

(7) WENZELL, WILLIAM T.
1855. ON CORYDALIS FORMOSA (OR TURKEY CORN). *In* Amer. Jour. Pharm., v. 27 (ser. 3, v. 3), p. 205–211.

PLATE I

A.—*Bikukulla cucullaria*, Dutchman's-breeches.
B.—*Bikukulla canadensis*, squirrel-corn.


B


A

FORCING THE GERMINATION OF FRESHLY HARVESTED WHEAT AND OTHER CEREALS[1]

By GEORGE T. HARRINGTON

Formerly Scientific Assistant, Seed-Testing Laboratories, United States Department of Agriculture

In the regions where the winter wheat harvest precedes sowing by only a few weeks, as in Wisconsin, farmers depend largely upon the current crop for fall sowing. The inability to secure a prompt and satisfactory germination test of the freshly harvested grain, when tested at the ordinary temperature, 20° C. or a little higher, has heretofore created an administrative difficulty, which became acute toward the end of the sowing season when a prompt report by the analyst was imperative. The work reported in this paper was done in an attempt to overcome this difficulty.[2] In all, about four dozen samples of wheat and one dozen each of barley and oats of the current crop, part of them gathered from the standing grain, were included in the investigation. More than half of these samples were gathered by hand in the State of Wisconsin. Others were sent there or to Washington from Colorado and from the Dominion of Canada.

Germination tests were made in 100-mm. Petri dishes with moist absorbent cotton as a seed bed, using only unbroken grains which showed no visible evidence of decay or injury. The majority of the samples were presoaked about half an hour in Gooch crucibles in running tap water, those badly infected with microorganisms being first sterilized from two to five minutes in a 1 per cent solution of silver nitrate. Only 50 grains were used in the majority of the tests, but 100 or 200 in some tests. Preliminary germination tests of all samples and a few special tests in which temperature effects were not important were made at room temperature (about 20° to 26° C.). Samples which germinated very well in the preliminary tests were not included in any of the tests with different methods of forcing germination.

On account of the limitations under which the work was done, it was necessary to count grains as germinating while in the very early stages of germination and to discontinue the majority of the tests at the earliest possible date. This practice was justified by observation upon further development at a favorable temperature for growth in a large number of selected cases.

Moisture determinations were made concurrently with the germination tests by drying small samples of the grain for two or three days in an oven in which the temperature was maintained at about 105° C. and the air was kept in circulation by a small electrically driven fan.

---

[1] Accepted for publication July 2, 1921.

[2] The greater part of the work was done in Madison, Wis., during August, 1919, with the cooperation of Prof. A. L. Stone, State seed inspector of Wisconsin, on whose suggestion the investigation was undertaken, Dr. W. W. Robbins, who at that time was with the Colorado Agricultural Experiment Station, and the Canadian analysts. Prof. Stone assisted in the arrangements for collecting samples in Wisconsin. Dr. Robbins and the Canadian analysts sent samples from their localities for test. The necessary equipment was furnished by the Branch Cereal Laboratory of the United States Department of Agriculture at Madison and the Department of Plant Pathology at the University of Wisconsin. After my return to Washington, Miss Bertha C. Hite, of the Seed-Testing Laboratories, continued the work, concerning herself especially with the effect of increased oxygen pressure and the germination of old cereals at different temperatures.

A great deal of work has been done by others on the after-ripening of seeds in general and more with the cereals than with any other class of seeds on account of their economic importance. Much of this work with the cereals by English and German investigators has been related to the adaptation of fresh grain for use in the brewing industry, although relations to agricultural practice and breeding have not been entirely neglected. Many and various hypotheses have been put forward to explain the process of after-ripening, but some of these lack convincing experimental evidence and none is of universal application.

RESULTS OF THE INVESTIGATION

EFFECT OF ARTIFICIAL DRY HEATING

Duchartre (*15*),[3] working with spring rye, wheat, and barley, was one of the first to call attention to the beneficial effect of artificial drying on the germination of seeds. He found that cereal seeds were capable of germination when they were still far from mature and their endosperms were just leaving the milk stage; that very young seeds germinated more slowly, but with as high a total percentage as the fully ripened grain; that artificial drying so affected these very young seeds that they germinated about as rapidly as the mature grain; and that such artificially dried young grain was satisfactory for seeding purposes.

Since Duchartre's publication, the New York (Geneva) Agriculture Experiment Station (*40*), Hotter (*24*), Hoffman (*22*, *23*), Hiltner (*21*), Atterberg (*6*), Kiessling (*28*), and Stapledon and Adams (*39*) are among those who have published results showing favorable effects upon germination from drying not after-ripened cereals and maize. Zade (*47*) found only slight advantage from artificial drying of wild oats when fresh, but when the dormant seeds which had been in moist sand for months were removed from the sand, dried, and remoistened a very large percentage germinated promptly.

Recently Kondo (*30*) has shown beneficial effects from drying not after-ripened rice. He considers this to be due to an increase in the oxygen supply made possible by a change in the oats. This explanation seems to be applicable to Zade's favorable results with wild oats, since Atwood (*7*) has shown that oxygen is the limiting factor in the germination of these seeds.

In nearly all the published work on artificial drying to hasten the after-ripening and germination of cereals the drying has been done by means of heating, and the results are therefore complicated by a possible effect of the heating per se. In fact Kiessling, in the article already cited, attempted to show that the beneficial effects he secured were really temperature effects and not effects of fluctuations in moisture content at all. However, Hiltner (*21*) and others have shown an accelerating effect upon after-ripening as a result of drying in vacuo or over sulphuric acid. The writer, in conjunction with Dr. William Crocker in work as yet unpublished, accelerated after-ripening of Johnson grass seed by drying over lime or over sulphuric acid, but the effect developed much more slowly and less completely here than when the seeds were heated in a drying oven. Apparently in this case both heating and drying are beneficial and the result seems to be related to the subsequent water intake of the embryo.

[3] Reference is made by number (italic) to "Literature cited," p. 97–100.

The effect of drying and heating treatment depends to an extent not always recognized upon the condition of the grain at the time of treating it, the temperature used, and the length of time the treatment is applied. Kiessling (*28*) found a beneficial effect upon barley in the early stages of after-ripening and a harmful effect in the later stages or after after-ripening was complete. Stapledon and Adams (*39*), with very mild dry heat treatments, secured beneficial results with good strong grain and harmful results with weak or diseased grain. Atterberg (*6*) reported a decrease in germinating capacity as a result of drying for a short interval, followed by an increase with longer drying. Wollny (*45*), working with fully afterripened cereals, found that artificial drying decreased germination but increased the productivity of the resulting plants. According to Worobiew (*46*) heating wheat before germination promoted a xerophytic type of structure and increased productivity under drought conditions but had no effect with ample moisture supply. Apparently Johannsen's failure (*25*) to induce the germination of fresh two-row barley and Stapledon and Adams' (*39*) only slightly favorable results with a number of cereals are to be explained as a result of drying for an insufficient length of time.

The temperatures used for drying have varied from about 30° C. to as high as 80° C., and the results with the higher temperatures have frequently been harmful on account of the relatively high moisture content of the cereals at the time the heating was begun. Müller (*36*), Waggoner (*42*), Harrington and Crocker (*20*), Nagai (*37*), Atanasoff and Johnson (*5*), and others have shown that cereal and other seeds are quite resistant to the effect of temperatures even as high as 100° C. if their moisture content is low when the heating commences. Zalenski (*48*) found 50° C. for a short time fatal to fresh green grain of winter rye and harmful to the grain in later stages of maturation.

Several have found a temperature around 40° C. to be best for increasing the germination capacity of not after-ripened cereals. Stapledon and Adams (*39*) used this temperature for three days with limited effect. Hotter (*24*) gave 10 days, Hiltner (*21*) 8 to 10 days, and Atterberg (*6*) 8 days as the length of time drying at 40° C. was necessary to bring not after-ripened cereals to their full germinating capacity. Atterberg obtained the same results from 8 days' drying at 30° C. if the seeds were dried in a current of air, a fact which seems to indicate that oxygen supply or rate of drying is important. Moritz and Morris (*34*) gave 43° C. as the maximum temperature to be used in kiln-drying barley for malting, and Hiltner stated that temperatures above 40° C. may be injurious to the fresh, relatively moist not after-ripened grain. Since barley is more resistant than the other cereals to the harmful effects of high temperatures 40° C. would seem to be as high a temperature as could safely be adopted for general use.

In the author's investigations heating for one day at 40° C. was without effect upon the germinating capacity of very moist, fresh wheat. Table shows the effects of other dry-heat treatments upon germination at room temperature.

Drying wheat eight days at 40° C. had a much more pronounced effect upon its subsequent germination than drying at the same temperature for five days. This drying treatment was more effective in hastening germination with barley than with wheat or oats, but it raised the germination capacity to nearly 100 per cent in all cases. When the temperature

was raised to 62° after eight days at 40°, and the drying was continued for one day, the rapidity and completeness of germination of oats and wheat were reduced to about the figures for the undried controls. Barley, after the same treatment, germinated even better than when dried only at 40°. This agrees with the statements of others that barley is more resistant to the injurious effects of high temperature than are the other cereals and in addition seems to indicate that up to the point at which injury begins, increasing the drying temperature would increase the effectiveness of the treatment.

TABLE I.—*Effect of artificial heating and drying*

Kind of grain and number of samples.	Drying treatment.	Average moisture content in percentage of wet weight.	Percentage of germination.			
			Average of all samples.		Samples showing greatest effect of the treatment.	
			2 days.	5 days.	2 days.	5 days.
Wheat, 3 samples	Control, not dried	12.3	[a] 61	69	[a] 52	6c
	Dried 5 days at 40° C.	8.1	71	78	68	76
Wheat, 4 samples	Controls, not dried	12.4	38	78	32	68
	Dried 8 days at 40° C.	6.9	59	95	54	98
Oats, 6 samples	Controls, not dried	11.6	20	85	8	62
	Dried 8 days at 40° C.	5.5	27	98	20	98
Barley, 3 samples	Controls, not dried	12.3	9	29	16	28
	Dried 8 days at 40° C.	6.1	71	99	86	[a] 100
Wheat, 2 samples	Controls, not dried	12.3	47	83	32	70
	Dried 8 days at 40° C.	7.0	68	93	54	88
	Same+1 day at 62° C.	4.8	38	73	24	50
Oats, 5 samples	Controls, not dried	11.6	20	83	8	62
	Dried 8 days at 40° C.	5.5	27	98	20	96
	Same+1 day at 62° C.	4.7	8	92	0	74
Barley, 1 sample	Controls, not dried	12.3	8	36	.....	.....
	Dried 8 days at 40° C.	5.7	58	100	.....	.....
	Same+1 day at 62° C.	1.7	80	100	.....	.....

[a] In 3 days.

Tests of several of the samples which showed poorest germination in five days were continued two days longer. Germination continued at a decreasing rate during this time, but was far from complete at the end of the time in case of some of the untreated lots, especially of barley and some of the lots of wheat and oats which had been heated at 62° C.

EFFECT OF PRESOAKING

Bleisch (*9*), with barley which germinated well without treatment, and Andrews and Beals (*3*), with fully after-ripened maize, have shown acceleration of germination as a result of presoaking. Hiltner (*21*) and Atterberg (*6*) greatly increased both germinating energy and germinating capacity of a number of kinds of freshly harvested cereals by presoaking combined with a pricking of the endosperms. Kiessling (*28*) decided that presoaking not after-ripened cereals at various temperatures had a uniformly harmful effect as compared with drying at the same temperatures and that this harmful effect was not entirely overcome by the

"heat stimulus," which he makes the basis upon which rests the beneficial results of both drying and presoaking. With water as the heating medium and the grain therefore in a sensitive condition the heat stimulus is felt in a few hours, whereas in dry condition days are required. Too long heating (overstimulation according to Kiessling (*28*), is of course harmful. Moufang and Vetter (*35*) therefore recommended soaking barley one hour at 45° C., followed by soaking in the cold in preparing it for malting. Too long-continued soaking at any temperature also is recognized as harmful by all who have worked upon the subject, and Wollny (*45*), Kidd and West (*27*), and others have emphasized the fact that harmful effects result from presoaking in an excess of standing water. This danger is reduced by using running water. According to Kidd and West (*27*), presoaking the red dwarf bean in excess of water increased the rate and vigor of germination but decreased the total percentage germinating and the number and dry weight of the seedlings which survived after three weeks in good soil. The injury thus measured and the amount of exosmosis from the seeds while soaking was greater when they were soaked at 5° to 10° C. or at 30° C. than when soaked at 20° C.

Braun (*10*) has recently described in connection with the treatment of cereals for seed-borne diseases a successful method of presoaking which is said to stimulate germination and early growth at the same time that it increases the efficiency of the sterilization treatments. Braun used a minimum quantity of water and limited the soaking time.

Wollny (*45*), Kraus (*31*), and Hiltner (*21*) reached conflicting conclusions, which Eberhart (*16*) attempted to harmonize, as to the effects of presoaking cereals and other seeds upon the resulting crop yield. All these except Hiltner (*21*) agreed that presoaking under certain conditions has a very marked effect in increasing crop yield. The seeds were sometimes sowed moist and sometimes after a period of drying, which did not seem to destroy the beneficial effects. Characteristic differences were observed at different stages of development between the plants from the controls and those from the treated seeds. Wollny (*45*) attributed the effects to coat changes which allow a more rapid water intake and initiate a more vigorous growth, though the percentage germinating is usually lessened, as Kraus (*31*) also decided. Eberhart (*16*) agreed that the permeability of the seed coats to water is increased by the presoaking as also by favoring ether treatments and that this increase is important; but he concluded that protoplasmic alterations also must be involved, since the effect is not temporary but persists throughout the growing period.

As already stated, the majority of the germination tests in this investigation were made after soaking the grain about one-half hour in running water. The grains to be tested for germination were placed in the bottoms of Gooch crucibles, which were then stacked in columns of not more than 12 in ring-stand supports. Tap water at approximately 18° C. from a spigot which gave a spattering stream, so that the water must have been well charged with atmospheric air as it bathed the grain, was then allowed to run through the entire columns. The object of this presoaking was to wash the seeds as free as possible of microorganisms and also to remove or precipitate (by means of chlorids in the water) the silver nitrate remaining on the grain in those tests in which the grain had been sterilized. There is no reason to believe that there were any harmful effects from this treatment.

One sample each of fresh wheat, oats, and barley was soaked in tap water at room temperature 1 hour and 5 hours in lots of 50 seeds each, which were then put to germinate at 16° and at 25° C. During the soaking the seeds were covered about 1 inch deep with water. The germination time was reckoned from the time when the soaking was begun. Table II shows the results of the germination tests.

One hour's presoaking greatly increased and 5 hours' presoaking slightly increased the germination within the first 31 hours, but presoaking even for 1 hour greatly reduced the percentage which germinated in 4 days in case of oats and barley but did not affect it in the case of wheat. The harmful effect of the presoaking was much greater when the subsequent germination test was made at 25° C. than when the germination temperature was 16°.

TABLE II.—*Effect of presoaking 1 and 5 hours in excess of standing water upon subsequent germination*

Kind of cereal.	Germination temperature.	Hours soaked.	Percentage germinating.	
			31 hours.[a]	4 days.
	°C.			
Wheat	16	0	0	100
		1	76	100
		5	12	96
	25	0	10	68
		1	16	70
		5	16	62
Barley	16	0	0	96
		1	46	68
		5	4	68
	25	0	4	76
		1	34	46
		5	8	18
Oats	16	0	0	98
		1	28	54
		5	26	58
	25	0	26	96
		1	26	34
		5	8	32

[a] Reckoned from the time the soaking was begun.

In order to get an idea of the progress of imbibition as affected by presoaking, 50 seeds of known weight of one poorly germinating sample each of wheat, oats, and barley were soaked one hour in small vials after which the water was poured off. The grain was emptied upon a blotting paper, the surface water was rapidly removed by rubbing with another small piece of blotting paper, and the percentage of increase in weight was determined in comparison with control lots on moist blotting paper in Petri dishes. The presoaked grain was then placed on moistened blotting paper in Petri dishes, from which it was removed from time to time and its increase in weight determined in comparison with the control lots. During the first eight or nine hours the grain was at room temperature, and thereafter in an incubator at 5° C. to prevent germination. Table III shows the percentage of increase in wieght of these lots of grain up to the end of the third day.

The rate of imbibition during the first hour was very much greater for the presoaked lots than for the controls. The presoaked oats continued absorbing water faster than the controls for several hours on wet blotters, while the presoaked wheat was absorbing water faster at the end of 72 hours than the control. With all three kinds of grain the difference in moisture content at the end of the presoaking period was practically maintained up to the end of the three days. There was scarcely any tendency for the moisture content of the soaked lots and the controls to become equalized within that time. This seems to indicate that the thorough wetting of the surface, displacing the surface film of air, in the case of the presoaked grain had established favorable conditions for the capillary movement of water over the surface of the grain toward the proximal region at which Schroeder (*38*) and Collins (*12*) have shown that nearly all water intake occurs and tended to increase permanently the imbibitional power of the presoaked grain. Wheat showed less difference between presoaked lots and controls than did barley or oats.

The high rate of water absorption during and immediately following the period of presoaking probably accounts for the early beginning of germination of presoaked grain, and undoubtedly the thorough filling of coat structures with unaerated water retards gaseous exchange, explaining the reduction in germination capacity resulting from presoaking in excess of water. It is, however, impossible to consider coat limitations to the total water-absorbing power as the cause of poor germination of the freshly harvested grain, since the quantity absorbed by even the control lots by the end of the third day must be far in excess of the minimum amount required for germination.

TABLE III.—*Percentage of increase in weight on wet blotters of grain presoaked 1 hour in comparison with controls not soaked* [a]

Number of hours.[b]	Percentage of increase in weight.					
	Wheat.		Oats.		Barley.	
	Presoaked.	Control.	Presoaked.	Control.	Presoaked.	Control.
1	15.7	8.9	38.7	13.1	25.4	8.4
2	21.3	13.4	46.7	16.1	32.2	15.7
4	29.5	23.3	59.8	26.1	42.3	28.4
8½	40.0	34.1	75.7	37.3	56.6	36.5
24	[c] 46.6	40.1	80.4	47.2	63.5	46.2
48	53.6	46.7	87.9	57.4	69.2	55.4
72	58.3	[c] 50.4	86.6	[c] 60.8	72.0	[c] 60.8
Moisture content after 72 hours (percentage of dry weight)	81.4	71.4	114.0	84.3	100.0	83.9

[a] Grain held for first 8 or 9 hours at room temperature, then at 5° C.
[b] Times given are only approximate for oats and barley, but the time was always the same for a presoaked lot and its control.
[c] One or more beginning to germinate.

EFFECT OF STERILIZATION WITH SILVER NITRATE SOLUTION

For use in the comparative germination tests at different temperatures to be discussed in a later section, grain of the samples which were badly infected with microorganisms—about one-half of the total number of

samples—was sterilized for five minutes in a 1 per cent solution of silver nitrate [1] with subsequent, thorough washing. Concurrent comparative germination tests of a number of samples with and without previous sterilization of the grain showed that the sterilization had no effect upon germination except to increase that of grain which molded badly without such sterilization.

Additional trials showed that wheat with not more than 10 to 12 per cent moisture could, with impunity, be immersed two minutes in a 1 per cent silver-nitrate solution even after cutting off the distal end, provided that the washing was very thorough. The cut surfaces of the endosperm subsequently darkened but germination did not suffer. A small percentage germinated even after the embryo had been scratched with a needle followed by two minutes in 1 per cent silver nitrate and thorough washing. Of course, a majority of the grains thus treated could not escape serious injury. This would be the case also with grains cracked or mutilated by thrashing or handling.

INFLUENCE OF THE SEED BED UPON GERMINATION

The result of the imbibition experiments and of the germination tests after presoaking suggested an attempt to secure by the use of a suitable germinating bed as much as possible of the accelerating effects of the presoak without its undesirable effects upon germinating capacity.

Trials were therefore made in 100-mm. Petri dishes with one rather poorly germinating sample each of wheat, barley, and oats, using seed beds of different character and moisture content.

For seed bed the following were used: (1) Four circular disks of heavy blue blotting paper; (2) raw cotton of low absorbing capacity; (3) Johnson & Johnson absorbent cotton. When blotting paper disks were used the grain was tested both on top of the four disks and between the second and third disks.

Four blotting paper disks weighed from 7½ to 8 gm. It required from 12 to 13½ cc. of water to saturate them and then 1 cc. additional made sufficient excess to drip when the uncovered Petri dishes were a little more than half inverted. This additional amount of water was all absorbed by the blotters in the next two or three hours. For the germination tests between blotters just the amount of water required to saturate the blotters was used in each case. For the germination tests on top of the blotters 1 cc. was added.

The Johnson & Johnson absorbent cotton in each dish weighed only 1.8 gr. (less than one-fourth as much as the blotters) and was saturated, when firmly pressed down, with 11 cc. of water. The addition of 6 cc. of water was then not sufficient to allow any to drip when the uncovered dish was a little more than half inverted. Thus the water-holding capacity of the absorbent cotton was greater than that of four times as great weight of the blotters; and of this large amount of water held by the cotton without flooding the seeds five or six times as much was readily available for rapid absorption by the seeds.

In the germination tests the amount of water required to saturate the cotton (11 cc.) was used in comparison with other tests using 2 cc. less and 6 cc. more water.

[1] A solution of silver nitrate is a very desirable medium for surface sterilization on account of the ease with which it can be removed or precipitated as the chlorid, the tenacity with which the silver chlorid is held on the coat structures, and the consequent relatively permanent sterility conferred.

The same weight of raw cotton was used as of the Johnson & Johnson cotton (1.8 gr. in each dish). Into this was carefully worked as much water as it could be made to hold, and the excess was then squeezed out and poured off. The amount retained could not be accurately determined but was about 10 cc. in each dish.

Table IV gives the result of the germination tests at 20° C. Fifty seeds were used in each test.

With barley and oats concurrent tests were made at 10°, 13° 17°, and 25° C. The best results, especially as to rapidity of germination, were obtained between saturated blotters and on top of the supersaturated absorbent cotton. Raw cotton and the only partially saturated absorbent cotton gave very poor results. In additional tests upon absorbent cotton using somewhat more than 17 cc. of water, the harmfull effects of flooding the grain with an excess of water became evident. The quantity used should not be enough so that any can be easily poured off from the Petri dishes, and the grains should be allowed to rest lightly upon the cotton instead of being pressed into it so that the water surrounds them.

The wheat was less sensitive to moisture conditions than the oats and barley, and the results were conflicting. Some other samples, tested at other times, seemed to be more sensitive than the one used in this series, and the optimal moisture conditions were the same as for oats and barley.

In germination tests at 10°, 13°, and 17° C., the percentage of germination varied less, according to seed bed and amount of moisture used, than at 20°, being between 90 per cent and 100 per cent in nearly all cases. The relation between moisture supply and rapidity of germination, however, was the same as at 20°, germination being most rapid between blotters and on top of supersaturated absorbent cotton and least rapid on raw cotton and partially saturated absorbent cotton. At 25° germination was very poor and the effect of moisture supply was greater than at 20°.

At all temperatures, the best root development was obtained between blotters and the most rapid epicotyl development on top of supersaturated absorbent cotton.

TABLE IV.—*Germination at 20° C. on different seed beds and with varying amounts of moisture*

Seed bed.	Cc. water.	Percentage germinated.								
		Wheat.			Barley.			Oats.		
		3 days.	5 days.	8 days.	3 days.	5 days.	8 days.	3 days.	5 days.	8 days.
On top of blotters	13–14.5	36	76	80	20	40	40	24	92	92
Between blotters	12–13.5	56	76	82	28	68	72	58	88	92
Raw cotton	[a] 10	22	84	92	4	32	32	16	76	76
J. & J. cotton	17	58	88	92	60	64	64	48	92	92
J. & J. cotton	11	46	88	88	16	52	52	24	72	84
J. & J. cotton	9	64	90	94	4	32	32	16	80	88

[a] About.

EFFECTS OF MECHANICAL TREATMENTS

1. WOUNDING

Hiltner (*21*), Atterberg (*6*), and Kiessling (*28*) improved the germination of various freshly harvested cereals by pricking or cutting the endosperms. Similar results were obtained by Zade (*47*) and by Atwood (*7*) with not after-ripened wild oats, by Harrington (*19*) with not after-ripened Johnson grass seed, and by Andrews and Beals (*3*) with fully after-ripened maize. Hiltner (*21*) and Atterberg (*6*) combined the wounding treatment with presoaking, to which the results were in part attributed. Various interpretations of the favorable effects of the wounding have been put forward.

In wild oats, Atwood (*7*) showed that the effect of the wounding is undoubtedly due to increased oxygen intake, oxygen deficiency being responsible for inability to germinate. Atwood's results with cultivated oats also indicate that increased oxygen supply may be involved in the effect with oats. Hiltner (*21*) believed that the wounding increases germination by allowing imbibition of sufficient moisture, and others have spoken of enzymic activity being initiated by the admission of oxygen and the oxidative transformation of proteins.

Kiessling (*28*) discredited all explanations of after-ripening which are based on coat exclusions. Since immediately closing the wounds with paraffine did not prevent the favorable effect of the wounding he believed that the effect of wounding is reached by exerting a "stimulus" upon the living protoplasm as in his interpretation of the effects of heat and cold on a stimulus basis. Behrens (*8*) came to the same conclusion, and Zade's dissertation also includes some data which might be thought to favor the idea that the dormant embryo of wild oats is in a state of exceedingly delicate equilibrium and responds readily to mechanical shocks. But in the case of Zade's work, it is probable that the rubbing of the embryo, which he interprets as causing mechanical stimulation of the embryo protoplasm, in reality increased the permeability of the coats to oxygen, thus supplying the increased amount of oxygen which Atwood later showed to be necessary. Furthermore, Kiessling's stimulus hypothesis is difficult to accept on account of the mechanical obstructions in the path of conduction to the embryo of any wound stimulus exerted upon the endosperm. The only path through the medium of living cells seems to be by way of the aleurone layer, which has no organic union with the embryo.

In the investigations herein reported two methods of wounding were adopted, both of which had been found to be very effective in causing the germination of dormant Johnson grass seed (*19*), namely, (1) cutting off the distal end of the grain just back of the embryo and germinating the embryo end alone, and (2) scratching the embryo itself along the entire length of one side of the scutellum by means of a bent dissecting needle. The axial organs were avoided, but the scutellum was rather deeply wounded. Only a few lots were treated in either of these ways.

When the distal ends were cut off and discarded the embryo ends of all lots of barley and oats and all but one lot of wheat so treated showed nearly complete germination at room temperature in from three to six days, but a marked tendency to decay after the treatment reduced the percentage of healthy seedlings from some unsterilized lots below that for the controls. One lot of wheat showed 100 per cent germination in

three days after treatment, against 56 per cent in three days and 64 per cent in five days in the control. The only lot which failed to germinate well after treatment was a lot of scarcely ripe wheat freshly harvested from the standing plants and carrying nearly 30 per cent (wet-weight basis) of moisture. This lot germinated 16 per cent in six days after treatment and failed to germinate at all in the control. Probably if comparable lots of oats and barley had been treated in the same way the germination would have been no better.

Simply removing the hulls from poorly germinating lots of oats and barley slightly increased their germination.

Scratching the embryo had much more effect than cutting off the distal end, inducing practically complete germination in two or three days of all lots thus treated except the one lot of wheat which was least affected by cutting of the distal end. This germinated 86 per cent in six days, while the control failed to germinate at all. The tendency to decay was much less than when the endosperms were removed, apparently on account of germicidal properties of the wounded embryos.

2. REMOVAL OF THE COAT STRUCTURES WITH CONCENTRATED SULPHURIC ACID

This treatment also has been found very successful with dormant Johnson grass seed (*19*). In this investigation it was used only with wheat. It is classed as a mechanical treatment because its effect apparently depends entirely upon the removal or weakening of the coat structures over the embryo. The grain was immersed in the concentrated acid for from 30 seconds to 5 minutes, washed in sodium-bicarbonate solution, rubbed free from as much as possible of the disintegrated tegumentary structures, and finally washed for about half an hour in running water. The first part of the coat to be visibly affected was that over the radicle, which became slightly charred in 30 seconds. After 3 minutes' treatment nearly the entire coverings rubbed off readily except over the edges of the scutellum and over the distal end of the caryopsis. Even after 5 minutes these parts were not wholly bared. The most rapid and complete germination was secured after 3 to 5 minutes' treatment, but the acceleration was quite noticeable when the treatments lasted only 30 seconds, therefore only slightly weakening the coat but probably effecting its permeability to a marked degree. This was the most effective of all the mechanical treatments, causing complete and prompt germination of the most resistant sample; but it is almost as tedious as the others and entails great danger of subsequent decay.

In all probability, the effect of all mechanical treatments here reported depends fundamentally upon the same cause, which may be either (1) relieving the axial parts of the embryo of some inhibiting substance by diffusion outward, or (2) an increase in respiration or an alteration in its nature by allowing more ready exchange of oxygen and respiratory products, or (3) a more obscure "stimulus" to the living protoplasm. Possibly, also, a similar stimulation of the epithelial layer of the scutellum plays at least a secondary part when the endosperm end of the caryopsis is removed or the scutellum is wounded by scratching. In this connection, it should be said that simply rubbing off the thin membrane which remained over the embryo of barley after the imbibed naked caryopses were removed from the scales also induced quick and complete germination of the most troublesome sample. The embryos were injured, however, sufficiently to cause some of the elongating coleoptiles to curve

abnormally upward on account of more rapid growth on the under than on the upper side. The possibility of a wound stimulus is therefore not eliminated.

GERMINATION AT DIFFERENT TEMPERATURES

Atterberg (*6*) was perhaps the first to call attention to the favorable effect of cool temperatures upon the germination of not after-ripened cereals. He considered after-ripening as simply the continuation of the normal process of ripening, and recommended that, with cereals too unripe to germinate well at from 13° to 15° C. the germination test be preceded by drying for eight days at 40° C. He also found that the use of alternating temperatures sometimes increased germination of such grain. Kiessling (*28*) got better germination at 12° to 16° C, than at 18° to 23° C, as late as November and better at 10° to 14° C. than at 18° to 22° C. until late stages of after-ripening, when the comparison was reversed. Recently, Stapledon and Adams (*39*) stated that from 12° to 15° C, gave best results with nearly all samples of fresh cereals. Of course at the low temperatures the rate of development is very slow and germination therefore has to be counted very "closely" or else the germination test has to be continued over a longer period.

An alternative is to transfer the grain to a higher temperature for an additional day or two as soon as practically all have begun to germinate.

Table V summarizes the results of a large number of germination tests at different temperatures.

The very favorable effects of temperatures well below 20° C. is strikingly evident. With oats and barley the highest average total percentages of germination in two weeks were secured at 5° and 9°. There were very considerable differences in response to temperature between individual samples of each kind of cereal.

TABLE V.—*Germination at different temperatures*

AVERAGE PERCENTAGE BEGINNING TO GERMINATE AT DIFFERENT TEMPERATURES

Kind of cereal.	Number of samples.	Number of days.	5° C.	9° C.	12° C.	16° C.	19° C.	22° C.
Wheat.....	16	2	5	54	89.5	89	88	52
		5	94	99	99	99	95	85.5
Oats.....	7	2	1	2	26	41	37	28
		5	32	69	79	71	65	55
		11–14	87	88	85	77	72	68
Barley.....	3	2	0	1	3	17	13	5
		5	28	63	67	63	45	24
		11–14	95	94	86	80	.....	.....

PERCENTAGE OF GERMINATION OF SAMPLES SHOWING MAXIMUM TEMPERATURE EFFECT

Wheat.....	1	2	0	0	50	40	22	8
		5	88	96	98	84	40	16
Oats.....	2	5	44	48	84	94	66	50
		5	82	94	92	68	52	48
Barley.....	1	5	20	80	86	80	56	20

A number of the poorer germinating samples of wheat were kept in the germinators for two weeks. With these, as with oats and barley, nearly all the grains previously ungerminated began to develop at the lower temperatures, but the germination of several samples remained far from complete at 19° C. and especially at 22°.

When barley and oats were kept in the cool germinators for the longer period, the endosperms usually became fluid and milky, while the later appearing germinations were often abnormal and weak. It is questionable, therefore, whether these should be counted as germinated. Atterberg's (*6*) combination of artificial drying with subsequent germination at cool temperature should be used with such samples. No such doubt is attached to the result with wheat, or to the early appearing germinations of barley and oats.

A temperature somewhat lower than 20° C. is of advantage also in making germination tests of older samples of cereals. Probably about 16° is the optimum. The percentage of germination of old samples frequently is slightly greater, and never is significantly less than at 20°, the development of the seedlings is sufficiently rapid, and the tendency to mold or decay is markedly less than at 20°. The results of this investigation show this to be true with oats and wheat, and incidental references in the literature on germination indicate that it is probably true also of barley and rye.

Several samples of spring wheat were very poorly developed on account of the rust epidemic, the selected grains for some samples being hardly more than half normal weight. Nevertheless, these samples responded as well to the mechanical treatments described in the previous section and to low temperatures as did the well-matured samples.

The use of relatively low germinating temperatures for winter cereals has the added advantage of simulating much more nearly the conditions under which the grain would germinate in the field. Planted in the fall, it has cool nights at least, and soon the average soil temperature, even in the day, is well below 20° C. Furthermore, Aderhold (*1*), Appel and Gassner (*4*), Gutzeit (*18*), Gassner (*17*), and Walster (*43*) have shown that the exposure of different kinds of seed or very young seedlings to low temperatures—always well below 20° C and frequently around 0°—has a very marked effect upon the subsequent development of the plant. In the case of cereals (especially winter cereals) this effect is decidedly beneficial, since it promotes an early, uniform, and abundant fruitage. The formative effect of temperature upon the plastic organs of plants has recently been further illuminated by Child and Bellamy (*11*). All of these investigations taken together open up a very interesting field for further study in connection with the after-ripening and germination of cereals.

EFFECT OF INCREASED OXYGEN PRESSURE UPON GERMINATION

As indicated in earlier pages Atwood (*7*) has found increased oxygen pressures efficient in forcing the germination of dormant wild oats, Kiessling (*28*) obtained similar results with tame oats and also found oxygen treatments helpful in increasing the germination of barley in the early stages of after-ripening but harmful in the later stages of after-ripening. Kondo (*30*) concluded that after-ripening in rice consisted of a process of oxygen storage and enzym formation and that the beneficial effect of drying was related to increase oxygen entrance. Hoffman

(*23*) elaborated a similar hypothesis, so far as oxygen is concerned, to explain the after-ripening of cereals.

On the other hand, Kiessling, in the article just referred to, showed that barley after-ripened more slowly in atmospheres in which two-thirds of the normal air was replaced by oxygen than in unaltered atmospheric air, and also that the beneficial effect of heating, either with or without simultaneous drying, was not dependent upon the partial pressure of oxygen during the period of heating. Takahashi (*41*), Nagai (*37*), and Akemine (*2*) have all shown that fully after-ripened rice germinates and develops considerably in entire or almost entire absence of oxygen, and Lukas (*33*) found that fully after-ripened oats and maize, as well as some other seeds, germinated more rapidly in reduced air pressure (therefore reduced partial pressure of oxygen) than in normal air. The behavior of rice is undoubtedly related to its ecology, this grain being adapted for germination under water, as are the seeds of many water plants (*13, 14*).

From Kiessling's (*28*) and Lukas's (*33*) results, it would seem either that the oxygen requirements of the embryos decreased during after-ripening, or else that increasing permeability of the coat structures during the after-ripening rendered oxygen in a given partial pressure more available for the use of the embryo. Atwood's (*7*) results show that the latter is probably the case with wild oats, while the work of the Japanese workers favors the former explanation with rice.

In this investigation, increased partial pressures of oxygen were used in testing the germination capacity of a number of not after-ripened samples of wheat. The apparatus used consisted of a small battery jar inverted within a larger battery jar so that two or three litres of gas was imprisoned over a water seal. The grain was tested on moist blotting paper in open Petri dishes floated on large corks within the small inverted battery jars, using 100 grains for each test. Increasing the percentage of oxygen in the atmosphere to 36 per cent had apparently the maximum effect on germination. With this percentage 85 per cent of one sample of wheat germinated in five days against 44 per cent with the normal 20 per cent of oxygen, while the amount of growth was nearly twice as great. With higher partial pressures of oxygen, the percentage of germination was about the same as with 36 per cent, but the rate of growth was less.

WATER CONTENT IN RELATION TO GERMINATION

Some of the earlier workers on after-ripening of cereals and the effect of artificial drying attempted to establish a relation between the moisture content of the seeds or alternations therein immediately preceding the germination test on the one hand and germination on the other hand. As shown in a previous section, artificial drying has usually had a beneficial effect. However, Atwood (*7*), Kiessling (*28*), Kondo (*30*) and others have shown in articles already cited that after-ripening takes place also when loss of water is prevented or even when more water is absorbed by the grain during the period of after-ripening. Besides the frequently favorable effect of presoaking as well as of drying is opposed to the view that the germinating capacity of not after-ripened grain is quantitatively related to its water content at the time the germination test is made. The relations involved go deeper, are less simple, and involve many other factors than moisture.

The author has found no quantitative relation between moisture content and germinating capacity. For instance, some samples of wheat collected from the shocks with 12 to 13 per cent moisture content germinated well at room temperature soon after collection, before their moisture content had fallen appreciably, while other samples collected and tested at the same time under the same conditions and with the same moisture content at the beginning of the test germinated poorly. Under proper treatment or after a period of after-ripening the latter germinated as well as the former.

In Table VI the germination data for a number of samples tested at the same time at room temperature are arranged according to the moisture content of the samples on moist-weight basis at the time the test was begun. Although there is a general falling off of germination with increasing water content, the water content groups overlap in regard to germination. All of the 11 samples falling in the last column (greater than 14 per cent moisture) were collected from the standing grain and tested within six days after collection. The relations between moisture content and germination are further illustrated by the data for individual samples in Table VII.

RATE OF AFTER-RIPENING: POSSIBLE VARIETAL DIFFERENCES

Attberg (*6*) stated that cereals may pass the entire winter in ordinary dry storage without becoming fully ripe (using "ripe" in the sense that he considers after-ripening as but a continuation of normal ripening processes), and that 1 or 2 months' drying at room temperature may be necessary. Kinzel (*29*) stated that oats reached their full germinating capacity in 2 months, but their full germinating energy only after 8 or 10 months.

TABLE VI.—*Germination of freshly harvested cereals of different moisture content in 5 days at room temperature*

	At moisture content of—				
	10 to 11 per cent.	11 to 12 per cent.	12 to 13 per cent.	13 to 14 per cent.	Greater than 14.
Wheat:					
Number of samples	8	6	9	6	6
Percentage of germination—					
Average	95	93	73	59	26
Maximum	100	100	100	98	52
Minimum	86	84	26	28	0
Oats:					
Number of samples	0	1	3	1	2
Percentage of germination—					
Average		70	75	48	64
Maximum			90		90
Minimum			52		38
Barley:					
Number of samples	0	0	1	2	3
Percentage of germination—					
Average			100	100	11
Maximum					16
Minimum					8

TABLE VII.—*Variety, length of time harvested, moisture content, and germination of wheat samples*

Sample No.	Variety.	Number of days since cutting.	Percentage of water.	Percentage of germination.
W_2	Winter	34	12.5	94 in 4 days.
W_3	do	34	13.4	86 in 4 days.
W_7[a]	Winter (Wisconsin No. 2)	34	13.3	42 in 5 days.
W_8[a]	do	34	12.8	56 in 5 days.
W_{11}	do	34	13.3	98 in 2 days.
W_4[a]	do	30	.....	28 in 5 days.
W_5[a]	do	30	13.4	40 in 5 days.
W_9	Spring (Kruger's) Wisconsin Wonder)	25	12.9	100 in 2 days.
5902	Winter (Turkey Red)	24	10.8	94 in 2 days.
5900	do	23	10.3	92 in 4 days.
W_6	Spring (Blue Ribbon)	17	13.2	52 in 5 days.
W_{10}	Spring (Marquis)	17	12.9	26 in 5 days.
W_{12}[d]	do	2	28.9	10 in 6 days.
W_{13}[b]	do	4	20.4	16 in 5 days.
W_{14}	Volunteer in oat field	4	15.0	52 in 5 days.
W_{15}	do	5	17.1	24 in 5 days.
W_{16}[c]	Spring	6	23.8	50 in 5 days.
W_{17}[c]	do	5	14.7	14 in 5 days.

[a] W_7 was from the outside and W_8 from the inside of the same bunch; W_4 from the top and W_5 from the bottom of the same bunch.

[b] W_{13} was collected from same field as W_{11} but was greener.

[c] W_{17} was from the same field as W_{16} but was standing and was somewhat better grains than W_{16}. W_{16} had been cut the preceding day.

[d] W_{12} to W_{17} were collected from standing grain on a rainy day and stored in the heads in heavy envelopes until the day of the test for germination.

Table VII presents such data as are available regarding rate of after-ripening arranged according to the length of time which had elapsed between cutting the grain or collecting the samples and the first germination test. The tests were made simultaneously at room temperature. On account of the lack of time, none of these samples were systematically retested at intervals to keep track of the rate of after-ripening.

There is no quantitative relation between the lapse of time since cutting and germinating capacity. The data suggest such varietal differences as have been described by Kiessling (*28*), who found striking differences, constant from year to year, in the initial dormancy and rate of after-ripening of different varieties and pure strains of wheat, oats, and barley and related these differences to differences in winter hardiness in case of winter wheat. Some varieties of barley after-ripened fully in two weeks, while others required more than two months. So far as the data in Table VII go, they indicated that Turkey Red winter wheat and especially Kruger's Wisconsin Wonder spring wheat are quickly after-ripening varieties; Marquis spring wheat and Wisconsin No. 2 winter wheat are slowly after-ripening varieties. According to Kiessling's (*28*) observations, the quickly after-ripening varieties, germinating sooner and more uniformly and becoming better established before the advent of cold weather, are also those recognized as the more hardy varieties. On this basis, Turkey Red ought to be more resistant to severe winters than Wisconsin No. 2, which is a strain isolated from Turkey Red stock. This condition is the reverse of what actually obtains in Wisconsin but is a matter which would undoubtedly vary in different localities and with grain from different sources. This suggested relation ought to be followed up in further investigations.

DISCUSSION

The work reported in this paper does not warrant an attempt to discuss in any detail the processes involved in after-ripening of the cereals or the numerous hypotheses previously offered to explain either these processes or the unanalyzed fact of after-ripening. Previous work by others has been cited when it was sufficiently related to the work herein reported. The enzymic theory of after-ripening has been almost entirely neglected in the references to the literature, not because this theory does not deserve mention but because the investigation did not touch on that phase of the general problem. The principal aim has been to present the results which are of value in the practise of seed testing and to fortify these results by citations of the results of others rather than to illumine or to explain the physiology of after-ripening or of germination.

All of the treatments which were of value in increasing the germination of the incompletely after-ripened cereals may logically be considered in connection with their oxygen and water requirements if we consider the effect of dry heating to be to increase the permeability of coat structures to oxygen. But there is no proof that heating has this effect. It may act in a more direct way on the embryo. In like manner in the other beneficial treatments effects upon the embryo protoplasm are not always out of the question. The bulk of the evidence does, however, seem to favor an explanation in which oxygen and water relations are involved and coat effects are important. Oxygen relations appear to be especially important. Since the cereals investigated seem under any conditions to absorb much more water during the germination test than the minimum required for their germination and since increased oxygen forced germination it seems probable that moisture and imbibition effects were related to the effects upon oxygen relations and respiration. Since the grain can be forced to germination and vigorous growth at any time, it does not seem that a storage of oxygen is a necessary part of after-ripening as postulated by Hoffman (*23*). The more probable explanation is that the treatments which force germination merely bring about the necessary increase in oxygen supply by removing coat structures or increasing their permeability to oxygen and that this change in permeability of the coat occurs also in normal after-ripening. Kiessling's (*28*) results with heating, with and without loss of moisture and in atmospheres of various compositions, upon which his stimulus hypothesis of the effect of heating is based are at least as readily explained on the basis of coat permeabilities. This is the situation which Atwood (*7*) found with wild oats, but it does not hold good for rice with which there must be either a storage of oxygen or a decrease in oxygen requirements during after-ripening.

Windisch (*44*), neglecting coat factors, lays emphasis on cell-wall permeability factors within the grain, which influence osmotic movements of nutrients. Windisch's hypothesis is not in consonance with the ready germination of the fresh grain when coat structures are removed or weakened.

The interrelations between moisture content, temperature, coat characteristics and changes therein, oxygen intake, rate of imbibition, rate and character of respiratory exchanges, response of the protoplasm to wounding and to the other treatments, changes in food reserves, the formation and activation of enzyms during the period of after-ripening and under both normal and forced germination ought to be thoroughly investigated, as well as the effects of the different treatments so far as

possible upon seedling vigor, upon the development of the resulting plant, and upon crop yield. The work of Kidd and West (*26*) in bringing together under the head of "Physiological predetermination" the result of previous work bearing on the latter subject, as well as the work referred to on previous pages, emphasizes the probability that the periods of maturation and germination of the seed, and perhaps also the intervening period of after-ripening, may be about the most impressionable stages in the life history of the plant for the reception of influences which are capable of modifying fundamentally the whole future course of its development. In future investigations along this line possible varietal differences should always be kept in mind.

SUMMARY

(1) Newly harvested cereals frequently do not germinate well at 20° C. or above under the ordinary conditions for germination tests. This fact has caused difficulty in administering State seed laws in the winter-wheat areas where harvesting precedes sowing by only a few weeks and the current crop is used for seeding.

(2) The embryos of the cereals investigated are never essentially dormant, dormancy being imposed by coat structures.

(3) Artificial dry heating, opening the coat structures over the embryo with incidental wounding of the scutella, and cutting off the distal ends, were effective in varying degree in inducing the germination of not after-ripened or partially after-ripened wheat, oats, and barley at room temperature.

(4) Removal or weakening of the coat structures over the embryos of wheat by the use of sulphuric acid was exceedingly effective in inducing complete germination in a minimum length of time.

(5) Artificial drying, to be completely effective, must be continued for a week or more and thus unduly delays the germination tests.

(6) Removal of the scales from oats and barley increased their germination somewhat, and removing the loose pericarp and tegumentary structures over dormant barley embryos caused complete germination at room temperature.

(7) Increased oxygen pressure in the atmosphere greatly increased the germination at room temperature of partially after-ripened wheat.

(8) The mechanical treatments, wounding and corrosion with sulphuric acid are very tedious processes and entail great danger of subsequent decay.

(9) Presoaking for even one hour in excess of water accelerated the germination of partially after-ripened wheat, oats, and barley but decreased the total germination. The soaking increased the rate of imbibition, and apparently the injurious effect upon total germination was the result of limiting gaseous exchanges by saturation of the surface layers of the grain with unaerated water. Presoaking half an hour in running aerated water did not seem to have any ill effect.

(10) For the germination of not after-ripened cereals, the seed bed should be such as to supply abundant water available for rapid absorption by the grain, but to do this without flooding the grain. Somewhat supersaturated absorbent cotton in Petri dishes satisfies these requirements if the grain is placed lightly on top of the cotton. Barely saturated blotters give equally good results if the grain is placed between the blotters, but not if the grain is placed on top of the blotters.

(11) A temperature considerably lower than 20° C. is much more satisfactory than 20° or higher for the germination of freshly harvested wheat, oats, and barley. Wheat responds to the low temperatures more uniformly than oats or barley. Nearly all fresh samples of wheat can be satisfactorily tested for germination at from 12° to 16° without previous treatment and without undue loss of time. With barley and oats the time required is longer, and it may sometimes be desirable to precede the germination test by dry heating.

(12) There is no quantitative relation between water content and germinability. After-ripening progresses at the same time as normal loss of water during curing of the grain, but not primarily as a result thereof. It occurs also at a modified rate when water loss is prevented or slight water absorption is caused.

(13) Some samples after-ripen much more quickly than others. The rates of after-ripening may be varietal characteristics and may be related to winter hardiness under some climatic conditions.

(14) Oxygen relations appear to be very important in the germination of not after-ripened cereals. The beneficial effects of mechanical treatments and of artificial drying and heating are probably related to increased oxygen supply to the embryo. It seems likely also that the permeability of coat structures to oxygen increases during after-ripening and that this increased permeability is related to the improved germination.

LITERATURE CITED

(1) ADERHOLD, Rudolf.
1906. ÜBER DAS "SCHIESSEN" DES KOHLRABIS. *In* Mitt. K. Biol. Anst. Land- und Forstw., Heft 2, p. 16–17.

(2) AKEMINE, Masao.
1914. ZUR KENNTNIS DER KEIMUNGSPHYSIOLOGIE VON ORYZA SATIVA (REIS). *In* Fühling's Landw. Ztg., Jahrg. 63, Heft 3, p. 78–93. Literatur, p. 93.

(3) ANDREWS, F. M., and BEALS, C. C.
1919. THE EFFECT OF SOAKING IN WATER AND OF AËRATION ON THE GROWTH OF ZEA MAYS. *In* Bul. Torrey Bot. Club, v. 46, no. 3, p. 91–100, 5 fig.

(4) APPEL, Otto, and GASSNER, Gustav.
1907. DER SCHÄDLICHE EINFLUSS DER HOHEN KEIMUNGSTEMPERATUREN AUF DIE SPÄTERE ENTWICKLUNG VON GETREIDEPFLANZEN. *In* Mitt. K. Biol. Anst. Land- und Forstw., Heft 4, p. 5–7.

(5) ATANASOFF, Dimitar, and JOHNSON, A. G.
1920. TREATMENT OF CEREAL SEEDS BY DRY HEAT. *In* Jour. Agr. Research, v. 18, no. 7, p. 379–390, pl. 48–49. Bibliography, p. 388–390.

(6) ATTERBERG, Albert.
1907. DIE NACHREIFE DES GETREIDES. *In* Landw. Vers. Stat., Bd. 67, Heft 1/2, p. 129–143.

(7) ATWOOD, W. M.
1914. A PHYSIOLOGICAL STUDY OF THE GERMINATION OF AVENA FATUA. *In* Bot. Gaz. v. 57, no. 5, p. 386–414, 13 fig. Literature cited, p. 410–414.

(8) BEHRENS, Johannes.
1907. UEBER DIE BEEINFLUSSUNG DER KEIMFÄHIGKEIT GEWISSER SAMEN DURCH NAKOSE UND VERWUNDUNG. *In* Ber. Landw. Vers. Anst. Augustenb., 1906, p. 60–64.

(9) BLEISCH, C.
1910. DER EINFLUSS HEISSER WEICHTEMPERATUREN AUF DIE KEIMUNG DER GERSTE. *In* Ztschr. Gesam. Brauw., Jahrg. 33, No. 45, p. 538–540.

(10) BRAUN, Harry.
1919. PRESOAKING AS A MEANS OF PREVENTING SEED INJURY DUE TO DISINFECTANTS AND OF INCREASING GERMICIDAL EFFICIENCY. *In* Science, n. s., v. 49, no. 1275, p. 544–545.

(11) CHILD, C. M., and BELLAMY, A. W.
1919. PHYSIOLOGICAL ISOLATION BY LOW TEMPERATURE IN BRYOPHYLLUM AND OTHER PLANTS. *In* Science, n. s., v. 50, no. 1294, p. 362–365.

(12) COLLINS, E. J.
1918. THE STRUCTURE OF THE INTEGUMENTARY SYSTEM OF THE BARLEY GRAIN IN RELATION TO LOCALIZED WATER ABSORPTION AND SEMI-PERMEABILITY. *In* Ann. Bot., v. 32, no. 127, p. 381–414, 9 fig. Bibliography, p. 414.

(13) CROCKER, William.
1907. GERMINATION OF SEEDS OF WATER PLANTS. *In* Bot. Gaz., v. 44, no. 5, p. 375–380.

(14) ——— and DAVIS, Wilmer E.
1914. DELAYED GERMINATION IN SEED OF ALISMA PLANTAGO. *In* Bot. Gaz., v. 58, no. 4, p. 285–321, 8 fig. Literature cited, p. 319–321.

(15) DUCHARTRE, P.
1852. NOTE SUR LA GERMINATION DES CÉRÉALES RÉCOLTÉES AVANT LEUR MATURITÉ. *In* Compt. Rend. Acad. Sci. [Paris], t. 35, no. 26, p. 940–942.

(16) EBERHART, Carl.
1906. UNTERSUCHUNGEN ÜBER DAS VORQUELLEN DER SAMEN . . . 95 p. Borna-Leipzig Inaug. Diss. Jena.

(17) GASSNER, Gustav.
1911. BEOBACHTUNGEN UND VERSUCHE ÜBER DEN ANBAU UND DIE ENTWICKLUNG VON GETREIDEPFLANZEN IM SUBTROPISCHEN KLIMA. *In* Jahresber. Angew. Bot., Jahrg. 8, 1910, p. 95–163, 6 fig.

(18) GUTZEIT, Ernst.
1908. VERSUCHE ÜBER DAS SCHOSSEN DER RÜBEN AND ANDERER PFLANZEN. *In* Mitt. K. Biol. Anst. Land- und Forstw., Heft 6, p. 20–23.

(19) HARRINGTON, George T.
1917. GERMINATION AND VIABILITY TESTS OF JOHNSON GRASS SEED. *In* Proc. Assoc. Off. Seed Analysts of No. Amer. 1916–17, p. 24–28.

(20) ——— and CROCKER, William.
1918. RESISTANCE OF SEEDS TO DESICCATION. *In* Jour. Agr. Research, v. 14, no. 12, p. 525–532.

(21) HILTNER, L.
1901. ÜBER DIE BESTIMMUNG DER KEIMFÄHIGKEIT VON FRISCH GEERNTETEN GETREIDESAMEN. *In* Mitt. Deut. Landw. Gesell., Jahrg. 16, Stück 32, p. 192–194.

(22) HOFFMANN, Friedrich.
1896. UEBER KÜNSTLICHE KORNAUSTROCKNUNG AUF DEM ENDLOSEN TUCH (SYSTEM ANGELE) IN DER STÄRKEFABRIK LOITZ. *In* Ztschr. Spiritusindus., Jahrg. 19, No. 49, p. 393–395.

(23) HOFFMANN, J. F.
1904. DIE EINWIRKUNG DES TROCKNUNGSPROZESSES AUF NACHREIFE UND AUSLESE (SELEKTION) BEIM GETREIDE. *In his* Das Versuchs-Kornhaus . . . p. 448–455. Berlin.

(24) HOTTER, Eduard.
1892. ÜBER DIE VORGÄNGE BEI DER NACHREIFE VON WEIZEN. *In* Landw. Vers. Stat., Bd. 40, p. 356–364.

(25) JOHANNSEN, Wilhelm.
1906. DAS AETHER-VERFAHREN BEIM FRÜHTREIBEN . . . Aufl. 2. 63 p., 13 fig. Jena. Einige Literaturangaben, p. 63–65.

(26) KIDD, Franklin, and WEST, Cyril.
1918–1919. PHYSIOLOGICAL PREDETERMINATION: THE INFLUENCE OF THE PHYSIOLOGICAL CONDITION OF THE SEED UPON THE COURSE OF SUBSEQUENT GROWTH AND UPON THE YIELD. II–V. REVIEW OF LITERATURE. *In* Ann. Appl. Biol., v. 5, no. 2, p. 112–142, 2 fig.; no. 3/4, p. 157–170, 220–251, 3 fig., 1918–19; v. 6, no. 1, p. 1–26, 3 fig., 1919. Bibliography, v. 5, p. 139–142, p. 169–170, 249–251; v. 6, p. 24–26.

(27) ———
1919. THE INFLUENCE OF TEMPERATURE ON THE SOAKING OF SEEDS. *In* New Phytologist, v. 18, no. 1/2, p. 35-39, 1 fig. Literature cited, p. 39.

(28) KIESSLING, L.
1911. UNTERSUCHUNGEN ÜBER DIE KEIMREIFUNG DER GETREIDE. *In* Landw. Jahrb. Bayern, Jahrg. 1, no. 6, p. 449–514.

(29) KINZEL, Wilhelm.
1900. ÜBER DIE KEIMUNG HALBREIFER UND REIFER SAMEN DER GATTUNG CUSCUTA. ÜBER DIE NACHREIFE VON HAFER. *In* Landw. Vers. Stat., Bd. 54, Heft 1/2, p. 133.

(30) KONDŌ, Mantarō.
1918. ÜBER NACHREIFE UND KEIMUNG VERSCHIEDEN REIFER REISKÖRNER (ORYZA SATIVA L.). *In* Ber. Ohara. Inst. Landw. Forsch. Kuraschiki, Japan, Bd. I, Heft 3, p. 361–387. Bibliographical footnotes.

(31) KRAUS, Carl.
1878. BEITRÄGE ZU DEN PRINCIPIEN DER MECHANISCHEN WACHSTHUMSTHEORIE UND DEREN ANWENDUNG. *In* Forsch. Geb. Agr. Phys., Bd. 1, p. 182–240.

(32) ———
1880–81. UNTERSUCHUNGEN ÜBER INNERE WACHSTHUMSURSACHEN UND DEREN KÜNSTLICHE BEEINFLUSSUNG. ÜBER DIE KÜNSTLICHE BEINFLUSSUNG DES WACHTHUMS DURCH VORQUELLEN DER SAMEN. *In* Forsch. Geb. Agr. Phys., Bd. 3, p. 275–287, 1880; Bd. 4, p. 59–62, 382–383, 1881.

(33) LUKAS, Franz.
1887. VERSUCHE ÜBER DIE KEIMUNG UND DAS WACHSTHUM VON PFLANZEN IN LUFTVERDÜNNTEN RÄUMEN. *In* Lotos Jahrb. Naturw., Bd. 35 (N.F. 7), p. 146–158.

(34) MORITZ, Edward Ralph, and MORRIS, George Harris.
1891. A TEXTBOOK OF THE SCIENCE OF BREWING . . . xvi, 534 p., 13 fig., 10 pl. London, New York.

(35) MOUFANG, Eduard, and VETTER, Louis.
1909. ZUR VERARBEITUNG VERREGNETER GERSTE. *In* Wchnschr. Brau., Jahrg. 26, no. 44, p. 571–573.

(36) MÜLLER, Gertrud.
1913. UNTERSUCHUNGEN ÜBER DIE VON WEIZENSAMEN UND WEIZENKEIMLINGEN ERTRAGENEN HÖCHSTEN TEMPERATUREN. *In* Ztschr. Pflanzenkrank., Bd. 23, No. 4, p. 193–198.

(37) NAGAI, Isaburo.
1916. SOME STUDIES ON THE GERMINATION OF THE SEED OF ORYZA SATIVA. *In* Jour. Col. Agr. Imp. Univ. Tokyo, v. 3, no. 3, p. 109–158, 2 fig., pl. 9. Bibliography, p. 156–158.

(38) SCHROEDER, Heinrich.
1911. ÜBER DIE SELEKTIV PERMEABLE HÜLLE DES WEIZENKORNES. *In* Flora, Bd. 102 (N. F. 2), Heft 2, p. 186–208, 4 fig.

(39) STAPLEDON, R. G., and ADAMS, Margaret.
1919. THE EFFECT OF DRYING ON THE GERMINATION OF CEREALS. *In* Jour. Bd. Agr. [Gt. Brit.], v. 26, no. 4, p. 364–381.

(40) STURTEVANT, E. Lewis.
1886. REPORT OF THE DIRECTOR. *In* N. Y. State Agr. Exp. Sta. 4th Ann. Rpt. 1885, p. 7–111.

(41) TAKAHASHI, T.
1905. IS GERMINATION POSSIBLE IN ABSENCE OF AIR? Bul. Col. Agr. Tokyo Imp. Univ., v. 6, no. 4, p. 439–442.

(42) WAGGONER, H. D.
1917. THE VIABILITY OF RADISH SEEDS (RAPHANUS SATIVUS L.) AS AFFECTED BY HIGH TEMPERATURES AND WATER CONTENT. *In* Amer. Jour. Bot., v. 4, no. 5, p. 299–313, 1 fig. Literature, p. 312–313.

(43) WALSTER, H. L.
1920. FORMATIVE EFFECT OF HIGH AND LOW TEMPERATURES UPON GROWTH OF BARLEY: A CHEMICAL CORRELATION. *In* Bot. Gaz., v. 69, no. 2, p. 97–126, 18 fig. Literature cited, p. 126.

(44) WINDISCH, WILHELM.
1905. WARUM KEIMT DIE GETROCKNETE BEZW. ABGELAGERTE GERSTE BESSER ALS DIE FRISCH GEERNTETE? *In* Wchnschr. Brau., Jahrg. 22, no. 7, p. 89–92, illus.

(45) WOLLNY, Ewald.
1885. SAAT UND PFLEGE DER LANDWIRTSCHAFTLICHEN KULTURPFLANZEN . . . xvi, 833 p., 38 fig. Berlin.

(46) WOROBIEW, S. J.
1915–17. THE EFFECT OF HEATING SEEDS UPON THE DEVELOPMENT OF THE PLANT; EXPERIMENTS MADE IN RUSSIA WITH WHEAT. Khozīaĭstvo, Yr. 10, No. 47/48, p. 1075–1083. Original in Russian, not seen. Abstract *in* Internat. Inst. Agr. [Rome] Internat. Rev. Sci. and Pract. Agr., v. 7, no. 4, p. 527–530. 1916. Also *in* Exp. Sta. Rec., v. 37, no. 8, p. 742. 1917.

(47) ZADE, Adolf.
1912. DER FLUGHAFER (AVENA FATUA). Arb. Deut. Landw. Gesell,. Heft 229, 91 p., 17 pl. (4 col.), 1 fold. map.

(48) ZALENSKI, R. G.
1916–18. POUVOIR GERMINATIF DU GRAIN DE SEIGLE D'HIVER RÉCOLTÉ PENDANT LES PÉRIODES DIVERSES DE SON GROSSISSEMENT ET DE SA MATURATION. *In* Zhur. Opytn. Agron. (Jour. Agr. Exp.), t. 17, kniga 6, p. 432–445, 3 diagr. In Russian. French resumé, p. 444–445. English abstract *in* Exp. Sta. Rec. v. 39, no. 5, p. 442. 1918.

A NEW AND EFFICIENT RESPIROMETER FOR SEEDS AND OTHER SMALL OBJECTS: DIRECTIONS FOR ITS USE[1]

By GEORGE T. HARRINGTON, *formerly Scientific Assistant, Seed-Testing Laboratories* and WILLIAM CROCKER, *formerly Plant Physiologist, Drug-Plant, Poisonous Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*

PREVIOUS RESPIRATION APPARATUSES

In the study of the dormancy, after-ripening, and germination of seeds, quantitative studies of their respiration are of major importance. For such studies on seeds and other small objects, a great many devices have been used, but none of them is perfectly adapted for the kind of studies the authors desired to make. Hence, the attempt to devise a new apparatus which would meet our requirements. Some of the apparatuses previously used, with their limitations, are indicated below. They are grouped according to the principle involved in the method.

(1) DEVICES FOR ABSORBING WITH CAUSTIC THE CARBON DIOXID (CO_2) RESPIRED INTO A STREAM OF CO_2-FREE AIR, OR OTHER GAS OR MIXTURE OF GASES, WHICH IS CONSTANTLY BEING DRAWN THROUGH THE APPARATUS CONTAINING THE RESPIRING MATERIAL WITH SUBSEQUENT DETERMINATION OF THE AMOUNT OF THIS ABSORBED CO_2 BY TITRATION OR BY FILTERING AND WEIGHING THE CARBONATE

Among the earliest, most widely known, and most generally used of hese devices are the long inclined tubes first used by Pettenkofer (*21*),[2] nd later somewhat modified by Pfeffer (*22*). These tubes, with or vithout modification, and combined with appropriate special devices or incubation of the experimental material, and for controlling the pres- ure when necessary, have been used by a large number of investigators n important studies on carbon assimilation and normal and intra- nolecular respiration. Winkler's modification in Hempel (*11*) of the Pettenkofer tube consists in bending it into the shape of a spiral so that t is easier to use.

Other devices which have been much used for the absorption of CO_2 n a stream of air which had been drawn through the receptacle containing he respiring material consist of vertical towers of caustic solution. The tower invented by Reiset (*23*) has three multiperforate disks of plati- um inserted to interrupt the stream of air and break it up into small droplets, thus insuring complete removal of the CO_2. Reiset tubes have been used by Brown and Escombe (*3*) in their important work on the energetics of green leaves, and by many others.

Recently, Gurjar (*7*) has adopted Truog's absorption tower, first used in soil analyses, for use in respiration studies. This tower consists simply of a vertical glass tube, ground into the neck of the flask which carries the caustic solution and filled to any desired height with glass

[1] Accepted for publication July 2, 1921.
[2] Reference is made by number (italic) to "Literature cited," p. 113-115.

beads which break up the current of air as it rises and take the place of the platinum disks of the Reiset tubes.

Sachs (in Grafe, *6*) drew the respired air through a series of simple absorption bottles, Kostytschew (*13*) used Giessler bulbs, and many other devices have been used by others.

With sufficiently large quantities of actively respiring material, apparatuses of this type are excellent for determining one side of the respiratory reaction. They have the advantage of avoiding the disturbing effects of changes in the partial pressures of oxygen and CO_2, but they neglect entirely the question of the amount of oxygen absorbed by the respiring material, which is quite as important as, and not always in proportion to, the amount of CO_2 given off.

Furthermore, they are not adapted for use with limited quantities of very small or relatively inactive seeds, as the amount of CO_2 given off in reasonable time may in this case fall within the limits of error of the method. Unless special precautions are observed, the experimental errors are likely to be rather large.

(2) ABSORPTION BY CAUSTIC SOLUTION OF THE CO_2 RESPIRED IN A CLOSED SPACE WITH OR WITHOUT SIMULTANEOUS MANOMETRIC MEASUREMENT OF THE OXYGEN ABSORBED

The apparatus used by Wolkoff and Mayer (*32*) was simply an inverted U-tube, one arm of which dipped into a dish of mercury, while the other arm was much enlarged and received the plant material and a dish of caustic solution. This end of the tube was, of course, closed during the experimental period, and the whole apparatus was immersed in a water bath.

Godlewski (*5*) cultured his material in a closed flask which contained a dish of caustic solution and was connected with a one-arm open manometer dipping into mercury. Stich (*24*) modified this apparatus for effective temperature control by immersion in a water bath.

When the manometer is used, this type of apparatus gives both oxygen consumption and CO_2 production, but since the CO_2 is absorbed as rapidly as it is given off and does not therefore compensate for the oxygen absorbed by the respiring material, the method often involves relatively large changes in pressure during an experimental period; these pressure changes might in some cases affect the results. Besides, like the preceding type of apparatus, this type requires special care to avoid errors due to the absorption of CO_2 from sources other than the respiration of the material being studied and is not adapted for use with limited quantities of very small material.

(3) INCUBATION IN A CLOSED SPACE, WHICH IS NOT SUPPLIED WITH EITHER CAUSTIC OR MANOMETER, WITH OCCASIONAL SAMPLING OF THE AIR FOR ANALYSIS IN SOME FORM OF MICRO-APPARATUS, OF WHICH THE BONNIER-MANGIN APPARATUS IS PERHAPS THE BEST. THIS APPARATUS AND ITS USES ARE DESCRIBED IN DETAIL BY THODAY (*26*). IT HAS BEEN WIDELY USED DURING THE LAST 35 YEARS

In the use of this method seeds have frequently been incubated for respiration in tubes inverted over mercury, but, as pointed out by one of us (*9*), the contact of the seeds with mercury may so alter their behavior as to make the use of this method entirely out of the question in specific cases.

Other methods of incubation without contact with mercury are, of course, possible. But when the mercury is used in contact with the respiring seeds, even if no effect of the mercury is known, this possibility should be carefully investigated before depending upon the results. At best the usefulness of the method is limited by the tediousness of the processes of incubation of the material and of sampling and analysis of the gas.

(4) MICRO-RESPIROMETERS, THE ESSENTIAL FEATURE OF WHICH IS THE USE OF VERY SMALL QUANTITIES OF GAS OR THE DETECTION OF VERY SLIGHT CHANGES IN ITS COMPOSITION

Thunberg's unnecessarily complicated apparatus (*27*), modeled on Peterson's older CO_2 apparatus, consists of two connected pipettes of similar size—one for analysis of the gas and the other for pressure compensation—each connected with a capillary tube and adjustable bulb of mercury. Thunberg's simpler apparatus, merely for demonstration of oxygen consumption (*28*), was modified by Winterstein (*31*), to allow the introduction of artificial atmospheres and further modified by Widmark (*30*) for accurate quantitative work with very small gaseous exchanges. As used by Widmark it is still too cumbersome for general use; one half of the apparatus serves merely as a compensator for the other half, in which the respiring material is incubated, and the results obtained are vitiated by the fact that oxygen consumption and CO_2 production are studied in duplicate lots of material instead of in identical material, or, if in the same material, then in alternate instead of identical periods. Besides, it seems probable that this apparatus could not be used when the gaseous exchange is as large as in most studies with moist seeds.

Tashiro (*26*) described two apparatuses which he claims will detect $\times 10^{-7}$gr. of CO_2 by absorption in a small drop of barium-hydrate solution. The author claims the possibility of accurate estimation of the amount of CO_2 by a series of trials in any given case. These apparatuses can not be used with as large quantities of CO_2 as are usually involved in respiration studies without extraordinarily large multiplication of experimental errors; they are relatively complicated and expensive; they take no account of the oxygen consumption; and in spite of the author's claim to the contracry, they seem to be adapted only to rather roughly approximate estimation even of the CO_2 given off.

Winkler, long ago, described a very sensitive chemical method for determining the percentage of oxygen in solutions, using alkaline potassium iodid, manganese chlorid, and hydrochloric acid. Recently, Osterhout and Haas (*20*) adapted this method for use in biological work with aquatic organisms. This method is at best too complicated for use when any other method is available. It is not adapted for use with other than aquatic organisms, and it neglects determining the CO_2 produced in respiration.

Warburg (*29*), by substituting a short column of hot *N/80* baryta water for the long column of the cold *N/10*, was able to determine small amounts of respired CO_2 with a maximum experimental error not exceeding 0.1 mgm. or 0.05 cc. CO_2.

Later, Krogh (*14*) developed a manometric apparatus which he described as "the simplest form of the closed-space respiration apparatus." Like Thunberg's apparatus and its various modifications, it makes use of

the principle of the compensating chamber to avoid the necessity of correcting for barometric and temperature changes. It is said to be sensitive enough to follow the oxygen absorption by a single insect egg weighing 2 mgm. in 10-hour periods. Krogh also described a later modification of Winterstein's apparatus which he says can be made even more sensitive than the Krogh apparatus.

Very recently, Lund (*16*) has developed a very simple apparatus for following the CO_2 production by small objects by titration of baryta water. With proper manipulation, it is very sensitive, but, like the other purely chemical methods, Lund's method requires special care to avoid errors due to the absorption of CO_2 from sources other than the respiring material, or errors from other sources. It also neglects oxygen consumption.

The range of some of the apparatuses of the micro type is sufficient to adapt them for much of the work on the respiration of seeds, but none of them provides for simultaneous determination of both sides of the respiratory exchange in the same material.

(5) INDICATOR METHODS

Doctor Haas (*8*) and Professor Osterhout (*17*) recently perfected an indicator method which is very sensitive in detecting small quantities of CO_2 respired. Their method is especially adapted for use in investigations in which only the comparative rate of production of CO_2 under different conditions is to be studied. It has the advantage of sufficient sensitiveness so that the experimental time can be very short, a matter frequently of prime importance; and the small amount of time required for the manipulation of the apparatus adapts the method for rapid and simultaneous determinations in several different experiments. By means of special calibrations and a sampling device the apparatus can be used for the approximate determination of the absolute amounts of oxygen absorbed and CO_2 produced; but this is not its special use. Indicator methods for the determination of the oxygen absorbed by the respiring material have also been described by Osterhout (*18*) and by Harvey (*10*), but these present certain grave objections and at best are not available for simultaneous determination of the CO_2 produced. The use of the indicator method for accurate measurement even of CO_2 output requires constant care to avoid errors due to buffer effects (*19*) or to the possible production of acid-reacting substances other than CO_2 or of alkaline-reacting substances.

In view of the limitations of the apparatuses previously used in investigations of respiration, we undertook to develop a new apparatus which would be simple, easy to use, and free from as many as possible of the objections which other apparatuses presented. With our apparatus, as described on the following pages, oxygen consumption and CO_2 production are determined in the same apparatus and for the same period of time, using the whole volume of air instead of a sample. Both sides of the respiratory exchanges are therefore followed in identical material and without multiplication of experimental errors. The gaseous exchanges are determined at the end of an experimental period by means of a manometer, which is an integral part of the apparatus, somewhat on the plan of Bunzell's oxidase apparatus (*4*). Since the CO_2 given off by the respiring material accumulates and tends to compensate for the oxygen absorbed by it no large changes in pressure occur during any

one experimental period. By making the pressure readings the basis of direct calculations both of oxygen consumed and of CO_2 given off the multiplication of operations and errors involved in a combination of manometric and analytic methods as used by Bonnier and Mangin (2) and by Aubert (1) is avoided. A number of different forms of apparatus embodying these principles have been constructed and tried, the one here described being finally adopted as giving most satisfactory results.

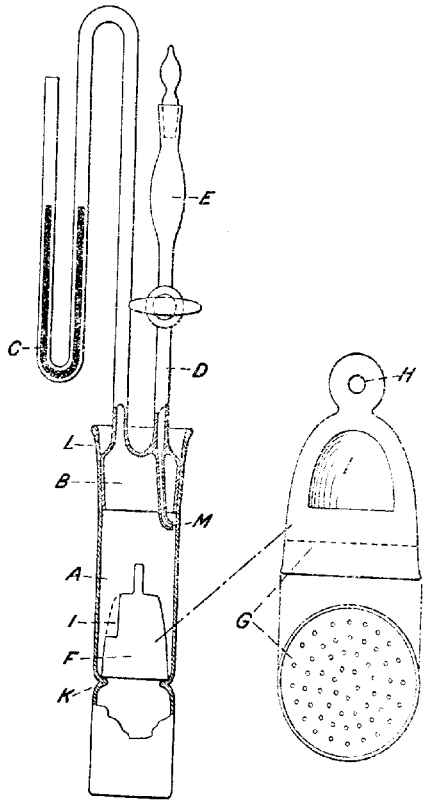


FIG. 1.—Drawing of a respirometer, large size (75 to 80 cc.).

DESCRIPTION OF THE APPARATUS

The apparatus (fig. 1) consists of (1) a cylindrical glass tube (A), in the form of a bottle, which is closed by a ground glass stopper (B), upon which are mounted a short open manometer of 2-mm. bore (C),[3] and an inlet or leveling tube (D) provided with a small chamber (E) for the reception of the caustic which is to be used for the absorption of the CO_2; and (2) a one-piece porcelain container (F) for the seeds.

[3] The bore of the manometers should be at least 2 mm. and may be 2.5, or 3 mm. If the bore is less than 2 mm., the mercury does not move freely enough to insure accurate work.

This seed container is in the form of an inverted crucible with a plate perforated with about 40 holes 1 mm. in diameter (G) inserted 5 mm. above the open base, and a ring at the top (H) by which, with the use of a bent needle or other suitable hook, to raise it or lower it in the tube (A). In one side of this inverted crucible is an opening (I), reaching from its top to 5 mm. above the perforated plate (G) and covering about 120 angular degrees.

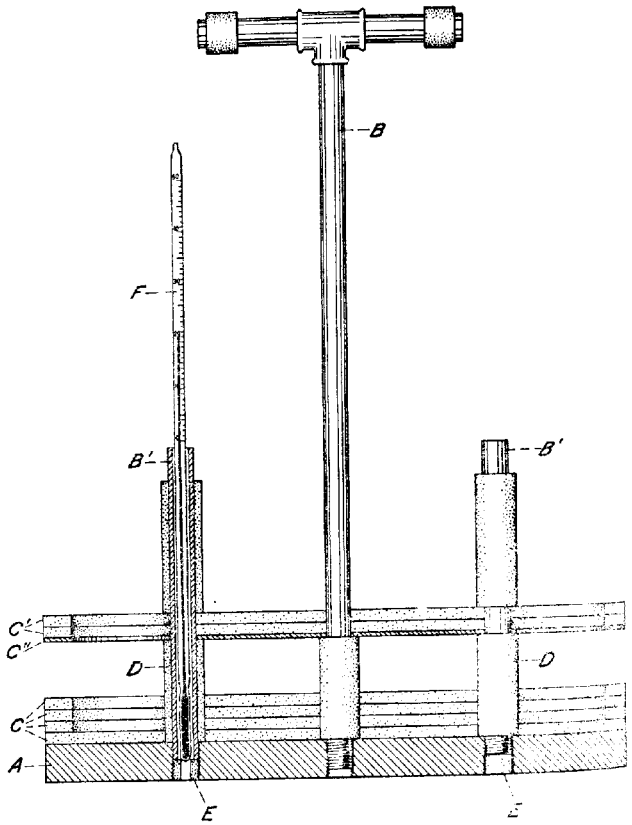


FIG. 2.—Median section of a carrier for eight respirometers.

The seed container rests upon nibs (K) pushed into the sides of the tube (A). The tall, slightly flaring top (L) of this tube provides for reinforcing the ground glass joint by a mercury seal.

We have used this apparatus in three sizes of about 20-cc., 40-cc., and 80-cc. capacity. The tube diameter of all three sizes is 3 cm., so that one size of seed container can be used for all. This simplifies their manufacture and makes them less expensive. With the smallest size, however, the nibs, which support the seed container in the other sizes, are replaced by a very low glass tripod, and the ring at the top of the

seed container has to be omitted. These small apparatuses are so short that the seed containers can be handled by inserting an appropriate hook into the cut-out which receives the seeds, whereas in the larger apparatuses the ring at the top is necessary.

The respiration apparatuses by themselves are hard to handle, so the carrier shown in median section in figure 2 was devised for use with any number up to eight at one time. Into threaded holes bored through a circular cast aluminum base (A) ½ inch thick and 9 inches in diameter are screwed nine posts, which consist of pieces of ¼-inch, heavy-walled brass tubing (B, B'). The tall central post (B) with the T connection serves as a handle, while to the other eight posts, covered with ½-inch thick-walled webb rubber tubing, the eight respiration apparatuses are bound with heavy rubber bands. A number of thicknesses of old rubber matting (C, C') appropriately placed, and all but the bottom one furnished with eight holes a little larger than the combined sizes of one of the brass posts and a respiration apparatus, hold the respirometers upright and protect them from the breakage which might be caused by bumping on metal.

The upper diaphragm of rubber matting (C') is stiffened by an underlying diaphragm of sheet aluminum or other appropriate material (C'') and is held at the proper height by short pieces of rubber tubing (D, D') on the brass posts. The holes in the bottom of the aluminum base are partially closed with small pure gum rubber tubing (E, E) and any one of the eight short hollow posts then serves for the reception of the thermometer (F). In case of fluctuations of temperature, this thermometer should not be depended upon to register the temperature of the water outside of the tubular post in which it is inserted.

OPERATION OF THE APPARATUS

The respiration tubes (fig. 1, A) having been provided with heavy rubber bands, the seed containers with their load of imbibed and sterilized seeds are lowered into place, and the contents of the apparatuses are rapidly aerated by drawing through each a strong current of air. The vasalined ground glass stoppers with the stopcocks opened and the caustic chambers unstoppered are rapidly put in place, forcing each down as far as it will go with a gentle twisting motion and placing them so that the bent delivery tubes (fig. 1, M) from the caustic chambers will deliver into the closed side of the seed containers. As soon as its top is in place, each apparatus is bound to its post. The whole battery is now submerged in the water bath, so that the water covers the tops of the respiration tubes, and the mercury is poured into these tops. As soon as the whole battery has come to the temperature of the water bath, which may be hastened by gently raising and lowering it, the stopcocks in the leveling tubes are closed, care being taken to see that the respirometers stand vertically and that the mercury is at the same level in the two arms of each manometer, the caustic chambers are stoppered, and the barometric pressure of the room is recorded.

After an appropriate length of time, which will usually be not less than 24 hours, from 1 to 3 cc. as required of 20 per cent sodium hydroxid (NaOH) is measured into each caustic chamber, leaving the stoppers out; the manometers are gently tapped to see that the mercury moves freely to its proper level; manometer readings are taken by holding a scale behind the manometer; the caustic is cautiously admitted into the

respiration tubes; a short interval, during which the barometer may be read, is allowed for complete absorption; and the manometers are again gently tapped and read.

From the manometer and barometer readings, the temperature used in the experiment, and the net volumes of the apparatuses,[4] corrected for the volumes of the respiring material and of the caustic which was admitted, and for the changed positions of the mercury in the manometers,[5] the three significant volumes of air under standard conditions (0° C. at 760-mm. pressure) can be computed. From these three volumes the volume of oxygen absorbed, the volume of CO_2 given off, and the respiratory quotient $\left(\frac{CO_2}{O_2}\right)$ are easily derived.

If extreme accuracy is desired, CO_2-free air may be introduced at the beginning[6] and further corrections may be introduced for the fact that the period for CO_2 production, from the putting of the apparatuses together to the making of the second manometer reading, is slightly longer than that for oxygen consumption, from the closing of the stopcocks to the time of the second manometer reading, and for the further fact that the volume determined for CO_2 respired includes also oxygen absorbed during the very short interval between making the first and second manometer readings.

Since the volume of oxygen absorbed is usually greater than that of CO_2 given off, the reduction of pressure thus brought about in the respirometer starts the flow of caustic when the stopcock is opened, and the absorption of CO_2 also aids in the introduction of the caustic. It is sometimes necessary, however, to force the last of the caustic down by gently blowing into the caustic chamber through a rubber tube slipped over its top.

The caustic, running down the side of the respirometer its entire length and over the top of the seed container, insures rapid absorption of the CO_2. Meantime, the roof of the seed container and the edge which protrudes below its perforated plate protects the seeds completely from contact with the caustic.

After a complete determination the battery of respirometers is removed from the water bath, stopcocks are opened, the mercury seals are removed by suction into a suction flask used as a trap, and the apparatuses are taken apart, emptied, and washed with a dilute acid and then with water. The seed containers may be washed by pouring acid over them without disturbing the seeds, and then both seeds and containers may be further washed under a stream of water by inserting them into Gooch crucibles in such a manner that the open sides of the containers are closed against the sides of the crucibles and the loss of seeds thus prevented.

The containers and seeds are now ready to be returned to the cleaned respirometers for another period of respiration.

---

[4] The net air capacity of each apparatus with the seed container in place is determined from the weight of the water it takes to fill it and is corrected for each experiment by subtracting the volumes of the respiring material.

[5] If the bore of the manometer tube is just 2 mm., a change of 3.185 mm. in the height of the mercury on the side toward the respiration chamber—that is, a difference of 6.37 mm. in the height of the mercury in the two arms of the manometer—corresponds to a change of 0.01 c. c. in the uncorrected volume of the air in the apparatus.

[6] The CO_2 in normal air would make a difference of only 0.2 mm. on the manometer, which is not much above the limit of error in adjustment and reading, and would, under the method adopted, affect the values for oxygen consumption and CO_2 production alike. It is believed that leaving a vessel of strong caustic solution exposed to the air for a few hours near where the apparatus is to be set up, and then aerating the apparatus and respiring material with air drawn from the open room over this caustic solution, reduces the error from atmospheric CO_2 well below the lowest possible limits of error. This is the procedure adopted in the work reported in the following article.

These respirometers are adapted for use with small material which does not have to be studied under aquatic conditions, provided it is not markedly sensitive to reduction in the partial pressure of oxygen or to increase in that of CO_2. Some seeds, however, are so small as to pass through the 1-mm. holes of the seed container. For use with such material, the perforations can be partially closed with paraffine. The change in the total pressure of all gases is always small. The manipulations and observations can be performed rapidly and the admittedly somewhat tedious computations may be delayed to suit one's convenience.

The apparatuses could probably be adapted for use with aquatic organisms, at least in certain cases, by substituting an appropriate container for the perforated seed container. They are probably not sufficiently sensitive for use in such work, as, for instance, that of Lund (*15*) with Paramecia, where the total differences in CO_2 production to be measured are sometimes such as would correspond to less than 1-cm. change in pressure as registered on the manometer of the smallest of the apparatuses here described, or about ten time the possible experimental error. In sensitiveness and therefore in the character of work for which they are adapted, they are intermediate between the apparatuses used for large objects and those of the micro type.

Plate 1 is a photograph of the carrier with two respirometers and a thermometer in place. To facilitate the reading of the manometers, which will almost invariably show some reduction in pressure, the respirometers were so constructed that their manometers are turned to the left when the stopcocks in the caustic chambers face outward or away from the tall central post.

The usefulness of the apparatus, as here described, can be greatly extended by attaching to the bottom of the respiration tube a side arm with stopcock for use in the introduction of artificial atmospheres. For work with artificial atmospheres it is desirable also to omit the short delivery tube (fig. 1, M) below the caustic chamber in order to facilitate the complete sweeping out of the original atmosphere. We have a few of the apparatuses in this modified form and have used them with good results, although they embody also some of the less desirable features of our earlier attempts.

The apparatus obviously can not be used except at approximately atmospheric pressure, though the proportions and nature of the gases present can be altered. For the study of respiration at greatly reduced or increased total pressures other forms of apparatus must be used. It will always be true, however, that the great bulk of the work on respiration will be done at atmospheric pressure.

FORMULAE FOR CALCULATION OF THE RESULTS OF RESPIRATION EXPERIMENTS

The following formulae can conveniently be used:

1. For the reduction of volumes to 0° C. and 760-mm. mercury pressure.

$$(1)\quad \text{Vol.}_{760} = \text{Vol.}_{t} \times \left(\frac{273\,P}{760\,(273+t)}\right).$$

$$= \text{Vol.}_{t} \times \left(\frac{0.3592\,P}{273+t}\right).$$

In which t and P represent the temperature of the bath in degrees centigrade and the pressure of the gas in millimeters of mercury. The

expression $\frac{0.3592}{273+t}$ represents a factor F which is constant for any given temperature. Substituting F in (1) gives:

$$(2)\quad \text{Vol.}^{\circ}_{760} = \text{Vol.}^{t}_{p} \times F \times P.$$

Values for F for different temperatures have been determined and are given here for convenience in calculations.

F for 0° C. = 0.001316
for 5° C. = 0.001292
for 10° C. = 0.001269
for 15° C. = 0.001247
for 20° C. = 0.001226
for 25° C. = 0.001205
for 30° C. = 0.001185
for 35° C. = 0.001166

Values for F for intermediate temperatures can be derived with sufficient accuracy by interpolation in the series given.

2. For changing from gas volumes to milligrams per gram dry weight per 24 hours.

Let a = number of cubic centimeters at 0° C. 760 mm.
b = weight in milligrams of 1 cubic centimeter at 0° C. 760 mm.
c = dry weight in milligrams of respiring material
h = respiration period in hours.

$$(3)\quad \left(\frac{24}{h}\frac{a}{}\frac{b}{}\right)\left(\frac{1{,}000}{c}\right) = x = \text{milligrams per gram dry weight in 24 hours.}$$

For oxygen, $b = 1.4289$; for CO_2, $b = 1.9768$.

By substitution of these values and the use of Y in place of one X, equation (3) becomes;

$$(4a)\quad X = \frac{24 \times 1.4289 \times 1{,}000\ a}{c\ h} = \frac{34293.6\ a}{c\ h} \text{ for oxygen.}$$

$$(4b)\quad Y = \frac{24 \times 1.9768 \times 1{,}000\ a}{c\ h} = \frac{47443.2\ a}{c\ h} \text{ for } CO_2.$$

3. For calculating temperature coefficients. Either of the formulae given by Kanitz (12) and reported by Denny may be used. Kanitz's second formula is $Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{t_2 - t_1}}$ in which K_2 represents the rate of any reaction at temperature t_2 and K_1 represents the rate of the same reaction at temperature t_1. The easiest form in which to use this equation in logarithmic solutions is

$$(5)\quad \text{Log } Q_{10} = (\text{Log } K_2 - \text{Log } K_1)\left(\frac{10}{t_2 - t_1}\right).$$

With the simplified equations (2), (4a), (4b), and (5) all of the computations can be easily performed.

RECORDING DATA AND CALCULATING RESULTS

Before beginning a respiration experiment the following data must be determined or provision made for its determination:

1. The volume of air in each apparatus without the respiring material (to 0.1 cc.).

2. Volume of the respiring material (to 0.1 cc.).
3. Net volume of air in the apparatus ($1-2$).
4. Weight of the respiring material (to 1 mgm.).
5. Percentage dry weight of the respiring material (this to be determined in material duplicating that which is used in the respiration experiment).
6. Dry weight of the respiring material from (5) (to 1 mgm.).

All necessary data must be carefully recorded at the beginning and end of each period of a respiration experiment. It is convenient to keep the data for each period in compact form on a 3 by 5 inch card or on a separate sheet of a 3 by 5 inch loose-leaf note book. The following form is suggested:

Exp. begun ..... ; Material .....
No. of period (1, 2, 3, etc.) ..... ; Temp. .....
Stoppers inserted .....
Stopcocks closed .....
Barometer ..... at ..... °C.; = ... at 0°C.
Manometers read ..... ; .....
Caustic used ..... ; Temp. .....
Barometer ..... at ..... °C. = ... at 0°C.
Manometer readings: 1 ..... ; 2 ..... ; 3 ..... ; 4 .....
Before adding caustic .....
After adding caustic .....
Remarks: .....
.....
.....
.....

The absolute net volume of an apparatus uncorrected for pressure or temperature is a base from which all other volumes are calculated, is uniform through any one set of calculations, and therefore need not be determined closer than to tenths of a cubic centimeter. On the contrary, the changes in volume of the gas it contains, corrected to standard conditions, are the actual data for respiratory exchanges. Inaccuracies approaching 0.1 cc. in determining these volume changes would sometimes seriously affect the results of an experiment. All volume computations should therefore be carried to hundredths of a cubic centimeter. For the same reason corrections of apparent volume for the changed positions of the mercury in the manometers should always be made. These corrections will frequently amount to more than 0.1 cc. The caustic solution used should be measured with all possible accuracy and should not be stronger than 20 per cent on account of the impossibility of accurate measurement of a stronger, more viscous solution. As indicated on a previous page, corrections may also be introduced for the short periods of time between the different operations.

Table I illustrates the calculation of gas volumes and units of respiratory exchanges (milligrams oxygen and CO_2 per gram dry weight of the respiring material in 24 hours) from data recorded at the beginning of an experiment and at the beginning and end of one experimental period. The data are taken from the first period of a respiration experiment with duplicate lots of 50 Newtown Pippin apple seeds in a very early stage of germination. The symbols in the left-hand margin of the table are used for convenient reference in later parts of the table to the quantities to which these symbols refer. The formulae, with their numbers, are taken from the preceding section. In practice the form in which these calculations are given can be considerably shortened.

TABLE 1.—*Calculation of gas volumes and units (milligrams per gram dry weight in 24 hours) of respiratory exchange*

Symbol.	Data.	No. 1.	No. 2.
	Volume of empty apparatus	42.1 cc.	44.0 cc.
	Volume of imbibed seeds	3.4	3.4
V	Volume of air in the apparatus	38.7 cc.	40.6 cc.
	Weight of the imbibed seeds	3,052 mgm.	3,112 mgm.
	Percentage of dry weight of the imbibed seeds	53	53
c	Dry weight of the seeds (calculated)	1,618 mgm.	1,649 mgm.
h_1	Stoppers inserted 12.30 p. m., May 27		
h_2	Stopcocks closed 12.45 p. m., May 27		
	Temperature 19.0° C.		
P_1	Atmospheric pressure 762.2 mm. at 22.5° C. = 759.3 mm. at 0° C.		
h_3, P_2	Manometer readings 10.00 a. m. May 29	+1.8	+1.3
V_2	Caustic solution added	1.0 cc.	1.0 cc.
h_4, P_3	Manometer readings 10.15 a. m. May 29	−23.5	−23.5
	Temperature 19.0° C.		
P_4	Atmospheric pressure 768.2 mm. at 24° C. = 765.0 mm. at 0° C.		
Formula (2)	Vol. $^{t}_{760}$ = Vol. $^{t}_{p}$ F. P.		
	$F = 0.001247 - \frac{4}{5}(0.001247 - 0.001226) = 0.001230$		
Vol. 1	$0.001230\ V_1P_1$	36.14 cc.	37.92 cc.
Vol. 2	$.001230\ V_1 + \frac{0.01P_2}{6.37}(P_4 + P_2)$	36.50 cc.	38.27 cc.
Vol. 3	$.001230\ V_1 - V_2 + \frac{0.01P_3}{6.37}(P_4 + P_3)$	34.35 cc.	36.08 cc.
Vol. 4	Approximate volume oxygen = Vol. 1 − Vol. 3	1.79 cc.	1.84 cc.
Vol. 5	Approximate volume CO_2 = Vol. 2 − Vol. 3	2.15 cc.	2.19 cc.
h	Length of period = $h_1 - h_1 = 45.75$ hours.		
	Period for oxygen = $h_1 - h_2 = 45.50$ hours.		
	Period for oxygen included with CO_2. = $h_1 - h_3 = 0.25$ hours.		
Vol. 6	True volume oxygen = Vol. $4 \times \frac{45.75}{45.50}$		
	Vol. 4	1.80 cc.	1.85 cc.
Vol. 7	True volume CO_2 = Vol. $5 - \frac{0.25}{45.50}$ Vol. 4	2.14 cc.	2.18 cc.
	Respiratory quotient = $\frac{\text{Vol. 7}}{\text{Vol. 6}}$	1.19	1.18
Formula (4a)	$X = \frac{34293.6a}{c\,h}$; a = Vol. 6	1.80	1.85
	c = Dry weight of seeds	1,618	1,649
	$h = 45.75$		
	X = mgm. oxygen consumed per gm. dry weight in 24 hours	.83	.84
Formula (4b)	$Y = \frac{47443.2a}{c\,h}$; a = Vol. 7	2.14	2.18
	c = Dry weight seeds	1,618	1,649
	$h = 45.75$		
	Y = mgm. CO_2 produced per gm. dry weight in 24 hours	1.37	1.36

Table II shows the calculation of temperature coefficients from an experiment with single lots of dormant York Imperial and Newtown Pippin apple seeds in successive periods at 13° and 30° C.

TABLE II.—*Calculations of temperature coefficients from data for successive periods at 13° and 30° C.*

	York Imperial.	Newtown Pippin.
Formula (5) $\text{Log } Q_{10}=(\text{Log } K_2-\text{Log } K_1)\left(\frac{10}{t_2-t_1}\right)$		
K_2 for oxygen consumption (units)	1.25	1.35
K_1 for oxygen consumption (units)	.65	.62
Log k_2	.09691	.13033
Log k_1	9.81291	9.79239
Difference	.28400	.33794
Log difference	9.45332	9.52884
$\text{Log}\left(\frac{10}{t_2-t_1}\right)=\text{Log}\left(\frac{10}{17}\right)$	9.76955	9.76955
Log (log Q_{10})	9.22287	9.29839
Log Q_{10}	.16706	.19879
Q_{10} for oxygen consumption	1.47	1.58
K_2 for CO_2 production (units)	1.47	1.51
K_1 for CO_2 production (units)	.59	.59
Log k_2	.16732	.17898
Log k_1	9.77085	9.77085
Difference	.39647	.40813
Log difference	9.59821	9.61080
$\text{Log}\left(\frac{10}{t_2-t_1}\right)=\text{Log}\left(\frac{10}{17}\right)$	9.76955	9.76955
Log (Log Q_{10})	9.36776	9.38035
Log Q_{10}	.23322	.24008
Q_{10} for CO_2 production	1.71	1.74

LITERATURE CITED

(1) AUBERT, Ephrem.
1892. RECHERCHES SUR LA TURGESCENCE ET LA TRANSPIRATION DES PLANTES GRASSES. *In* Ann. Sci. Nat. Bot., sér. 7, t. 6, no. 1, p. 1–90, 9 fig. Reprinted with slight change in title as Pt. I of his Thèse, Paris. 1892.

(2) BONNIER, Gaston, and MANGIN, Louis.
1884. RECHERCHES SUR LA RESPIRATION ET LA TRANSPIRATION DES CHAMPIGNONS. *In* Ann. Sci. Nat. Bot., sér. 6, t. 17, p. 210–305, pl. 17–20. Bibliographical footnotes.

(3) BROWN, Horace T., and ESCOMBE, F.
1905. RESEARCHES ON SOME OF THE PHYSIOLOGICAL PROCESSES OF GREEN LEAVES, WITH SPECIAL REFERENCE TO THE INTERCHANGE OF ENERGY BETWEEN THE LEAF AND ITS SURROUNDINGS. *In* Proc. Roy. Soc. [London] ser. B. v. 76, no. 507, p. 29–111, 1 fig.

(4) BUNZEL, Herbert H.
1914. A SIMPLIFIED AND INEXPENSIVE OXIDASE APPARATUS. *In* Jour. Biol. Chem., v. 17, no. 3, p. 409–411, 1 fig.

(5) GODLEWSKI, Emil.
1882. BEITRÄGE ZUR KENNTNISS DER PFLANZENATHMUNG. *In* Jahrb. Wiss Bot. [Pringsheim], Bd. 13, Heft. 3, p. 391–543, 1 fig.

(6) GRAFE, Viktor.
1914. ERNÄHRUNGSPHYSIOLOGISCHES PRAKTIKUM HÖHEREN PFLANZEN. X, 494 p., 186 fig. Berlin.

(7) GURJAR, A. M.
1917. THE ADAPTATION OF TRUOG'S METHOD FOR THE DETERMINATION OF CARBON DIOXIDE TO PLANT RESPIRATION STUDIES. *In* Plant World, v. 20, no. 9, p. 288–293, 1 fig.

(8) HAAS, A. R.
1916. A SIMPLE AND RAPID METHOD OF STUDYING RESPIRATION BY THE DETECTION OF EXCEEDINGLY MINUTE QUANTITIES OF CARBON DIOXIDE. *In* Science, n. s. v. 44, no. 1125, p. 105–108.

(9) HARRINGTON, George T.
1917. FURTHER STUDIES OF THE GERMINATION OF JOHNSON GRASS SEEDS. *In* Proc. Assoc. Offic. Seed Analysts No. Amer., 1916/17, p. 71–76.

(10) HARVEY, E. Newton.
1919. THE RELATION BETWEEN THE OXYGEN CONCENTRATION AND RATE OF REDUCTION OF METHYLENE BLUE BY MILK. *In* Jour. Gen. Physiol., v. 1, no. 4, p. 415–419.

(11) HEMPEL, Walther.
1892. METHODS OF GAS ANALYSIS. Translated from the second German edition by L. M. Dennis. XV, 384 p., 101 fig.

(12) KANITZ, Aristides.
1915. TEMPERATUR UND LEBENSVORGÄNGE. IX, 175 p., 11 fig. Berlin. Bibliographical footnotes. Review by F. E. Denny *in* Bot. Gaz., v. 62, no. 2, p. 156–157. 1916.

(13) KOSTYTSCHEW, S.
1908. ÜBER DIE ANTEILNAHME DER ZYMASE AM ATMUNGSPROZESSE DER SAMENPFLANZEN. *In* Biochem. Ztschr., Bd. 15, Heft 2, p. 164–195. Bibliographical footnotes.

(14) KROGH, August.
1916. THE RESPIRATORY EXCHANGE OF ANIMALS AND MAN. VIII, 173 p., 35 fig. (partly on pl.). London, New York, etc. Bibliography, p. 151–168.

(15) LUND, E. J.
1918. QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION. IV. THE RATES OF CO_2 PRODUCTION BY STARVED AND FED PARAMECIA, AND THEIR POSSIBLE RELATION TO THE RATES OF OXIDATION IN THE UNFERTILIZED AND FERTILIZED SEA URCHINS EGG. *In* Amer. Jour. Physiol., v. 47, no. 3, p. 318–327. Bibliography, p. 327.

(16) ———
1919. A SIMPLE METHOD FOR MEASURING CARBON DIOXIDE PRODUCED BY SMALL ORGANISMS. *In* Biol. Bul., v. 36, no. 2, p. 105–114, 2 fig. Bibliography, p. 113–114.

(17) OSTERHOUT, W. J. V.
1918. A METHOD OF STUDYING RESPIRATION. *In* Jour. Gen. Physiol., v. 1, no. 1, p. 17–22, 1 fig.

(18) ———
1918. AN INDICATOR METHOD OF MEASURING THE CONSUMPTION OF OXYGEN. *In* Jour. Gen. Physiol., v. 1, no. 2, p. 167–169.

(19) ———
1918. THE DETERMINATION OF BUFFER EFFECTS IN MEASURING RESPIRATION. *In* Jour. Biol. Chem., v. 35, no. 2, p. 237–240, 1 fig.

(20) ——— and HAAS, A. R. C.
1917. AN ADAPTATION OF WINKLER'S METHOD TO BIOLOGICAL WORK. *In* Jour. Biol. Chem., v. 32, no. 2, p. 141–146, 1 fig.

(21) PETTENKOFER, Max.
1862. UEBER EINEN NEUEN RESPIRATIONS-APPARAT. *In* Abhandl. Math. Phys. Cl. K. Bayer. Akad. Wiss., Bd. 9, Abt. 2 (Denkschr., Bd. 34), p. 231–276.

(22) PFEFFER, W.
1885. ÜBER INTRAMOLEKULARE ATHMUNG. *In* Untersuch. Bot. Inst. Tübingen, Bd. 1, Heft, 4, p. 636–685, 1 fig.

(23) REISET, J.
1880. RECHERCHES SUR LA PROPORTION DE L'ACIDE CARBONIQUE DANS L'AIR. Deuxième note. *In* Compt. Rend. Acad. Sci. [Paris], t. 90, no. 20, p. 1144–1148, illus.

(24) STICH, Conrad.
1891. DIE ATHMUNG DER PFLANZEN BEI VERMINDERTER SAUERSTOFFSPANNUNG UND BEI VERLETZUNGEN. *In* Flora, Jahrg. 74 (N. R. 49), Heft 1, p. 1–57, 2 fig.

(25) TASHIRO, Shiro.
1913. A NEW METHOD AND APPARATUS FOR THE ESTIMATION OF EXCEEDINGLY MINUTE QUANTITIES OF CARBON DIOXIDE. *In* Amer. Jour. Physiol., v. 32, no. 2, p. 137–145, 2 fig.

(26) THODAY, D.
1913. ON THE CAPILLARY EUDIOMETRIC APPARATUS OF BONNIER AND MANGIN FOR THE ANALYSIS OF AIR IN INVESTIGATING THE GASEOUS EXCHANGES OF PLANTS. *In* Ann. Bot., v. 27, no. 107, p. 565–573, 2 fig.

(27) THUNBERG, Torsten.
1905. EIN MIKRORESPIROMETER. EIN NEUER RESPIRATIONSAPPARAT, UM DEN RESPIRATORISCHEN GASAUSTAUSCH KLEINERER ORGANE UND ORGANISMEN ZU BESTIMMEN. *In* Skand. Arch. Physiol., Bd. 17, Heft 1/2, p. 74–85, 1 fig.

(28) ——
1905. EIN EINFACHE ANORDNUNG, UM DIE SÄUERSTOFFZEHRUNG KLEINERER ORGANISMEN ODER ORGANE ZU DEMONSTRIEREN. *In* Zentbl. Physiol., Bd. 19, No. 10, p. 308–310.

(29) WARBURG, Otto.
1909. MASSANALYTISCHE BESTIMMUNG KLEINER KOHLENSÄUREMENGEN. *In* Ztschr. Physiol. Chem., Bd. 61, Heft 3, p. 261–264, 2 fig. (on 1 pl.).

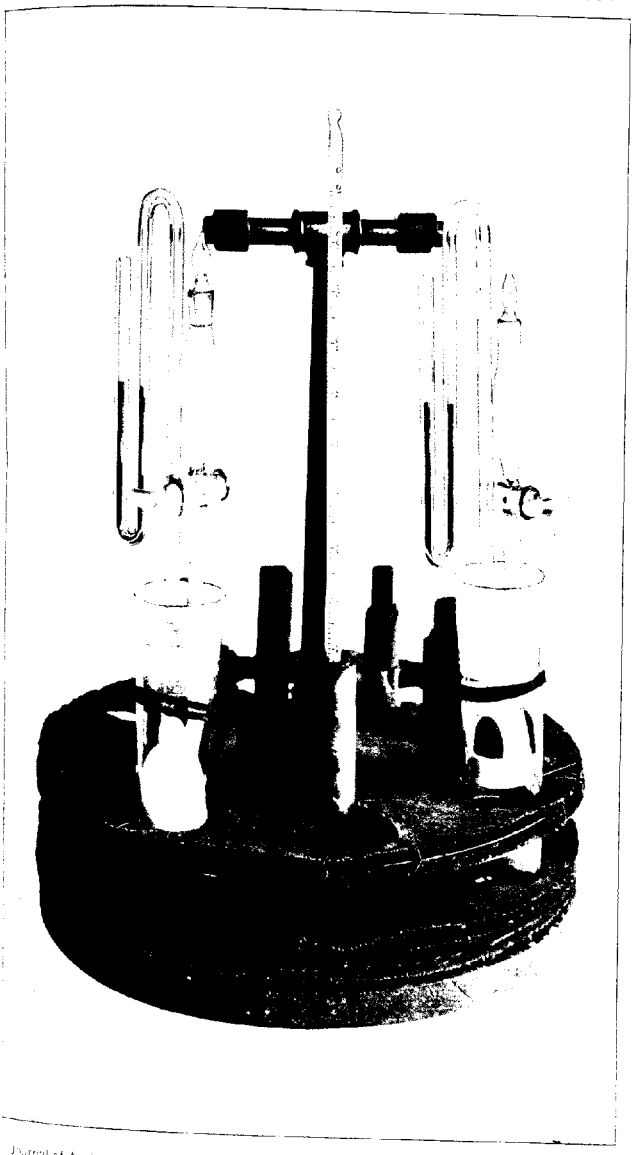
(30) WIDMARK, Erik M. P.
1911. ÜBER DIE HANDHABUNG DES THUNBERG-WINTERSTEINSCHEN MIKRORESPIROMETERS NEBST EINIGEN DAMIT AUSFÜHRBAREN VERSUCHEN FÜR DES PHYSIOLOGISCHE PRAKTIKUM. *In* Skand. Arch. Physiol., Bd 24, Heft 5/6, p. 321–338, 2 fig.

(31) WINTERSTEIN, Hans.
1907. UEBER DEN MECHANISMUS DER GEWEBSATMUNG . . . *In* Ztschr. Allg. Physiol., Bd. 6, Heft. 3/4, p. 315–392, 4 fig. Bibliographical footnotes.

(32) WOLKOFF, A. von, and MAYER, Adolf.
1874. BEITRÄGE ZUR LEHRE ÜBER DIE ATHMUNG DER PFLANZEN. *In* Landw. Jahrb., Bd. 3, p. 481–527, 4 fig.

PLATE 1

Photograph of the carrier shown in figure 2, with two large respirometers and a thermometer in place.



RESPIRATION OF APPLE SEEDS[1]

By GEORGE T. HARRINGTON

Formerly Scientific Assistant, Seed-Testing Laboratories, Bureau of Plant Industry, United States Department of Agriculture

This study was made, using the manometric respiration apparatuses and following in detail the procedure described in the previous article (*11*) on a new and efficient respirometer for use with seeds and other small objects.

The size of apparatus used in each case was determined by the number and relative activity of the seeds to be studied. The results have been computed in terms of milligrams of oxygen consumed and milligrams of CO_2 given off per gram dry weight of the respiring seeds in 24 hours. The temperature coefficients have been computed for both processes for each temperature interval used, whenever the seeds used were in sufficiently stable condition to make it desirable to do so.

FIRST EXPERIMENT.—Newtown Pippin seeds from fruit stored at 0° C. until May 7. Seeds removed in the next few days and incubated on moist blotters at about 5° to 10° C. Experiment begun May 20 with six parallel lots of seed at temperature of 19°; 25 or 50 seeds per lot at beginning of experiment. (Table I.)

TABLE I.—*Respiration of Newtown pippin seeds at 19° C.*

Period of respiration.	Number of lots.	Condition of seeds.	Gaseous exchanges (mgm. per gm., dry weight) per day.		CO_2/O_2 (volume)
			Oxygen consumed.	CO_2 produced.	
Hours.					
18¼	6	Intact	1.09	1.30	0.86
43	2	Intact; 26 per cent germinating at end of period	.83	1.01	.87
	4	Outer coats removed; 67 per cent germinating at end of period	[a] 2.35	[a] 2.73	.....
			[b] 3.28	[b] 3.81	.84
21	1	Intact; many germinating at end of period	.97	1.26	.94
	1	Intact; germinated at beginning of period	3.42	3.97	.84
	2	Outer coats removed; all germinating at end of period	[a] 2.97	[a] 3.15	.....
			[b] 4.14	[b] 4.39	.77
	1	Outer coats removed; germinated at beginning of period	[a] 7.48	[a] 6.83	.....
			[b] 10.43	[b] 9.53	.66

[a] Based on dry weight of intact seeds.
[b] Based on dry weight of seeds with outer coats removed.

[1] Accepted for publication July 2, 1921.

Journal of Agricultural Research,
Washington, D. C.

Vol. XXIII, No. 2
Jan. 11, 1923
Key No. G-215

The results after the first period show the effects upon respiration of removing the outer coats[2] and of germination. When the outer coats were removed, the results were computed on the basis of calculated dry weight after removal of these coats as well as on the basis of the dry weight of the intact seeds. Some seeds were discarded during the experiment on account of advancing germination or to leave a convenient number in a given condition for use in a given apparatus.

The respiratory quotient was always less than unity, as should be expected when the material being respired is fatty or oily, and therefore poor in oxygen, as is the case with apple seed.

Removing the outer coats approximately doubled or trebled the respiratory intensity without altering the respiratory quotient until germination had well begun. Connected with this increased respiration of the seeds was an acceleration of germination, though these seeds were sufficiently after-ripened so that the majority of them germinated in four days even in intact condition.

As germination advanced, the respiratory intensity rapidly increased, and in seeds freed from their outer coats the respiratory quotient became lower in value. (See last two entries in last column of Table I.) In intact seeds at a later stage of germination there is a similar decrease in the value of the respiratory quotient, not shown here but illustrated in other experiments.

Godlewski (*9*),[3] Bonnier and Mangin (*4*), Gerber (*8*), and Ivanoff (*13*), have reported low values for the respiratory quotient of germinating oily seeds, or young seedlings.

According to Godlewski, the respiratory quotient of fatty seeds during swelling is a little less than 1.0, but the value of the quotient falls as soon as the roots appear and is maintained about constant at from 0.55 to 0.65 during the period of greatest respiratory intensity, after which it rises to about 1.0. His value for the period of greatest intensity is slightly lower than the lowest shown in Table I. According to this author, and to Gerber, the respiratory quotient of germanating starchy seeds is constantly about unity during the entire period of germination.

Bonnier and Mangin, however, found that with both oily and starchy seeds during germination the respiratory quotient is at first near unity, falls gradually to a minimum variable with the species, and then rises again to its initial value. In this connection, it should be borne in mind, that even starchy seeds contain more or less oil. In endospermous, starchy seeds, this oil is usually concentrated in the embryo. If, as would be expected, this oil is drawn upon very early during germination, the large use of oxygen in its conversion into sugars would give a temporarily low value to the respiratory quotient of such seeds, exactly as these authors point out in regard to oily seeds. They conclude that, if there are characteristic differences in the respiratory activity of the two classes of seeds, these differences consist only in the minimum value of the respiratory quotient, and not in the fact or direction of its variation

[2] The coverings of the mature apple seed consist of: (1) a thick brown, fibrous outer coat with open micropyle; (2) a thin, translucent inner coat of very dense structure and without openings, suggesting semipermeable characters; and (3) a delicate, whitish, cellular tissue, somewhat thicker than the inner coat, and evidently endospermous. Layer 3 is closely adherent to layer 2, and it is impossible to remove the two separately, though the embryo is easily bared by removing the two together. The outer coats constituted about 33 per cent, the inner coats and endosperm about 11 per cent, and the embryos about 56 per cent of the wet weight of the seeds. The dry weights of different parts were about 15 per cent, 5 per cent, and 33 per cent of the original wet weight of the whole seeds.

[3] Reference is made by number (italic) to "Literature cited," p. 129-130.

Gerber gives a minimum value of about 0.30 for the respiratory quotient of germinating oily seeds, while Ivanoff reports values as low as 0.13. Gerber argues from his results that if the particular fats stored in any given seed are easily oxidized (flax) the respiratory quotient will be near that for complete oxidation (0.70), while if they are difficultly oxidizable (Ricinus) the quotient will be nearer that for simple conversion of the fat to sugars and therefore relatively low.

Table I also shows a tendency for the value of the respiratory quotient to rise temporarily in the initial stages of germination of intact apple seeds. Gerber (*8*) points out a similar condition when the radicles are unable to break the coats, and assumes, without proper evidence, that his occasional high values of the respiratory quotient (slightly greater than 1.0) in such cases are due to the transformation of sugars into alcohol in the "imprisoned and fatigued" cells. The same result would be obtained by the splitting up of the carboxyl groups of organic acids—a reaction, which, like the formation of alcohol, might result from a deficiency of oxygen in the respiring tissue. This oxygen deficiency, in turn, might occur as a result of the limiting effects of coat structures relatively impermeable to oxygen. But it is also true that a rise in the respiratory quotient until it becomes nearly equal to 1 might result from the oxidation of previously accumulated sugars more rapidly than the fats are broken down, without any reference to oxygen deficiency. Irwin (*12*) also calls attention to the theoretical possibility of obtaining a high production of CO_2 as a result of the splitting of carbonates and bicarbonates in the respiring tissues by accumulating organic acids. Since free oxygen would ordinarily be consumed in the formation of the organic acids, this oxygen consumption would tend to balance the CO_2 produced by such a breaking up the carbonates and to hold the respiratory quotient at about its previous value. In Irwin's work the increased production of CO_2 by Salvia petals as a result of etherization was accompanied by decreased acidity, and a simultaneous increase in oxygen consumption indicated that the increased CO_2 production resulted from true respiratory exchanges. It is regrettable that Irwin's apparatus (the Haas-Osterhout indicator apparatus (*19*) and technic did not involve the simultaneous determination of oxygen consumption and CO_2 production so that the respiratory quotient could be determined and closer deduction could be drawn as to the probable origin of the increased output of CO_2. In the absence of such data, it is natural to suppose that reduction in acidity at the same time that CO_2 production was increased would involve an oxidation of organic acids and an increase in the respiratory quotient.

SECOND EXPERIMENT.—Seeds from a cider press mash, not germinated after incubation as follows:

Lot A, 50 days at 20° C. in intact condition, then 68 days at 20° with outer coats removed.[4]

Lot B, 50 days at 30° C. in intact condition, then 68 days at 20 to 30°, daily alternation, with outer coats removed.[4]

Lot C, 50 days at 20° C. in intact condition, then 68 days in an ice box at about 5° to 10°, with outer coats removed.[4]

The experiment was begun May 27, 1919, 25 seeds in each lot.

All the seeds in lot C germinated within the first two periods of two days each at 19° C. During the first period the volume of gaseous ex-

[4] See footnote 2 on page 118.

changes was about twice and during the second period about four times that for lots A and B, which remained dormant. The respiratory quotients for the two periods were 0.81 and 0.75—a little higher than for lots A and B.

Table II shows the respiratory activity of lots A and B at different temperatures during a period of two months. During this time 12 per cent of the seeds germinated and were discarded, while a few others were discarded on account of injury. All results were calculated on the basis of the dry weight of the material actually present in the apparatus whether this included the inner coats or these had been removed.

Both the respiratory intensity and the respiratory quotient rose and fell as the temperature of the experiment was raised and lowered. For each temperature, there was a fairly definite respiratory quotient which was reestablished each time the seeds were brought back to that temperature from another temperature, either higher or lower.

TABLE II.—*Respiration of dormant cider press apple seeds with outer coats removed*

Period	Temperature.	Gaseous exchanges (milligrams per gram dry weight per day).				CO_2/O_2 (volume)	
		Oxygen consumed.		CO_2 produced.			
		A[1]	B[2]	A[1]	B[2]	A[1]	B[2]
Hours	°C						
45	19	1.02	1.35	0.98	1.19	0.69	0.64
46	19	1.00	1.61	.99	1.63	.72	.73
46	19	1.33	1.61	1.36	1.52	.74	.69
21	30	2.97	2.94	3.23	3.07	.79	.75
46	30	3.20	2.56	3.39	2.44	.77	.69
45	30	4.23	2.93	4.73	3.03	.81	.75
43	19	2.86	2.83	2.61	2.64	.67	.67
45	19	2.80	2.53	2.57	2.24	.66	.64
47	19	2.77	1.39	2.48	1.25	.65	.65
25	30	5.59	2.05	5.67	1.74	.73	.61
48	30	4.40	1.95	4.70	1.85	.77	.68
48	19	3.25	1.81	3.00	1.69	.67	.67
118	19	3.31	1.28	3.12	1.16	.68	.65
115	10	1.60	.98	1.37	.76	.62	.56
121	10	1.64	1.06	1.40	.86	.62	.59
118	10	1.55	.97	1.35	.77	.63	.58
194	0	.43	.38	.12	.21	.21	.40
140	13	1.81	1.07	1.72	.95	.69	.64
49	30	4.47	3.30	4.91	3.27	.79	.72
INNER COATS REMOVED.[3] INCIPIENT GERMINATIONS BY END OF PERIOD							
20	30	19.45	16.74	22.91	17.87	.85	.77
ALL GERMINATING BY END OF PERIOD							
43	19	10.09	8.86	10.30	9.61	.74	.78

[1] Lot A was previously incubated under germination conditions at 20° C., 50 days in intact condition, then 68 days with outer coats removed.

[2] Lot B was previously incubated under germination conditions, 50 days at 30° C. in intact condition, then 68 days t 20 to 0° C with outer coats removed.

[3] See footnote 2 on page 118.

Lot B showed a generally lower respiratory intensity, lower respiratory quotients, and a smaller change in respiratory quotient resulting from any given change in temperature than did lot A. All of these differences seem to indicate that lot B was in a state of deeper dormancy than lot A. This condition probably reflects a greater impoverishment of lot B in the materials used in respiration as a result of previous incubation at a higher temperature. Both the greater intensity of respiration and the higher respiratory quotient at the higher temperature would work in this direction.

This conception of the relation of dormancy to respiratory exchanges follows closely the observations of Lund and others. Lund (*16*) has shown a great reduction in respiratory intensity of Paramecia as a result of partial starvation, or, in other words, impoverishment in respirable materials. The character of his material would naturally lead one to expect a much greater reduction in respiratory intensity on account of such impoverishment than would occur in dormant seeds, whose respiration is already on a rather low plane. In a later paper (*17*) the same author has shown that loss of irritability by Planaria under anaerobic conditions is much more closely associated with decreased CO_2 production than with absence of consumption of free oxygen. In the seeds of lot B, a decrease in CO_2 production relative to oxygen consumption under aerobic conditions seems likewise to correspond to a state of greater quiescence or deeper dormancy.

Lot A increased in intensity of respiration at a given temperature, perhaps as a result of the temperature changes. Lot B, which had previously been given a regular daily alternation of temperatures, showed a greater intensity of respiration than lot A during the first few periods of the experiment, increased temporarily, and then fell to the original intensity. The effect of alternating temperatures upon the respiration of dormant seeds is, however, unknown, and may bear no relation to the differences here noted.

Ziegenbein (*23*), working with germinated seeds, decided that sudden changes of temperature cause no modification of respiratory intensity to either extreme of the alternation. On the other hand Palladine (*20*), using similar material which had been previously cultivated on sugar solutions at different temperatures, concluded that passing from a high temperature (36° C) or from a low temperature (7°) to a medium temperature (18° to 20°) stimulates the respiration of plants. Blanc (*3*) objected to Palladine's technic and, in a very careful piece of work upheld Ziegenbein's view. Blanc concluded that for each temperature there is a definite respiratory intensity, and the transition from the respiratory activity corresponding to a given temperature to that corresponding to a different temperature is made gradually allowing for all respiratory activities intermediate between those of the extreme temperatures.

While Blanc's results seem conclusive for the material he worked with, neither he nor the previous workers used material which was in a dormant condition. In view of the known effect of temperature alternations upon the germination of many kinds of seeds it seems possible that the respiration of dormant seeds may at least in some cases be affected by such alternations in ways quite different from any effect upon actively growing material. This point needs investigation. My own results as here given, while suggestive, certainly furnish no proof of such effects.

The very low respiratory quotient at 0° C. is particularly interesting because it suggests storage of oxygen at low temperatures as at least one cause of the favorable effect of low temperatures upon after-ripening. Even at 19°, the weight of oxygen taken in was greater than that of CO_2 given off, so that unless some of the excess oxygen was split off as water, the seeds were actually gaining in dry weight. The obvious suggestion here is that oxygen is being used in the transformation of fats to sugars or in the formation of organic acids. Sugars and acids have been shown by Applemann (*1*), Atwood (*2*), Brannon (*5*), Eckerson (*7*), and Jones (*14*) to accumulate during storage of dormant structures at low temperatures. Eckerson (*7*), in fact, showed an increase in acidity of haw and apple seeds during incubation at low temperatures, which were favorable for after-ripening. I have found that the after-ripening of apple seeds takes place also in commercial cold storage when the seeds remain within the intact fruit. While perhaps it should not be expected in this case that oxygen would be available for storage in the seeds, still the volume of the respiratory exchanges of the seeds within the fruit must be small and must be governed more or less by the respiratory activity of the surrounding pulp, so that the maintenance of a low respiratory quotient, with concurrent increase in the acidity of the seeds, may not be out of the question even here.

Possibly also the increase of oxidizing enzyms during after-ripening at low temperatures, as shown by Eckerson for apple seeds, and by Crocker and Harrington (*6*), Jones (*14*), and Rose (*21*) for other seeds, and the fact that their catalase activity at least is exceedingly high immediately after germination, as I have found in work as yet unpublished, and as previously reported by Crocker and Harrington (*6*) and by Jones (*14*) for other seeds, are significantly related to their respiratory exchanges, especially to storage of oxygen and low respiratory quotient during after-ripening at low temperatures and to intense oxidation with high respiratory quotient in the first stages of germination. The correspondence between respiratory activity and catalase activity is especially interesting in view of previous work by Applemann (*1*), Crocker and Harrington (*6*), and Kohl (*15*) which seems to connect this enzym definitely with respiratory processes.

In the last two periods of the experiment, the naked embryos were used. Germination began at once, and in less than three days all had germinated There was the customary tremendous increase in respiratory intensity. There was also a slight increase in the respiratory quotients above those which were normal for the dormant seeds at the temperatures used, indicating that, in this early stage of germination, oxygen-rich substances were being respired more rapidly than the fats were being broken down to their component sugars. During the last period the oxygen in the respiration apparatuses, as computed from their known volume, was quantitatively used up, and the respiratory quotients were probably somewhat higher than they would otherwise have been.

The temperature coefficients for oxygen consumption and for CO_2 production in consecutive periods of the preceeding experiment are given in Table III.

Table III shows values for Q_{10} ranging from scarcely more than 1 to nearly 4, with two exceptional higher values for CO_2 production at low temperatures in case of lot A. The values are greater for lot A than for lot B, emphasizing again the difference in response of the two lots to

external conditions. They are also greater for CO_2 production than for oxygen consumption, greater when changing from 19° to 30° C., than when the reverse change is made, and greater at low temperatures than at higher temperatures. The differences in temperature coefficients, when the reciprocal changes 19° to 30° and 30° to 19° are made, suggest a stimulating effect of the higher temperature, which is not entirely lost upon return to the lower temperature.

Upon removal of the inner coats and the onset of rapid germination, with vigorous respiration, the temperature coefficients remained about the same as for the same temperature interval with dormant seeds.

TABLE III.—*Temperature coefficients (Q_{10}) for the respiration of dormant apple seeds with outer coats removed*

Temperature interval.	Q_{10} for oxygen consumption.		Q_{10} for CO_2 production.	
°C.	A[1]	B[2]	A[1]	B[2]
19 to 30	2.08	1.73	2.20	1.90
30 to 19	1.43	1.03	1.71	1.14
19 to 30	1.89	1.43	2.12	1.35
30 to 19	1.32	1.07	1.51	1.09
19 to 10	2.32	1.35	2.46	1.53
10 to 0	3.63	2.53	11.04	3.60
0 to 13	3.03	2.21	7.66	3.15
13 to 30	1.70	1.59	1.85	2.07
30 to 19 naked germinating embryos	1.95	1.78	2.07	1.76

[1] Lot A was previously incubated under germination conditions at 20°C., 50 days in intact condition, then 68 days with outer coats removed.
[2] Lot B was previously incubated under germination conditions, 50 days at 30°C. in intact condition, then 68 days at 20° to 30° with outer coats removed.

THIRD EXPERIMENT.—Newtown Pippin seeds. Fruit stored at 0° C. to May 7, seeds removed during next few days and incubated under germination conditions at 5° to 10° for 13 to 18 days. Many seeds had germinated. Only those not germinated were used for the respiration experiment. Fifty seeds in each lot at beginning of experiment. Some discarded from time to time on account of advancing germination or to keep a convenient number in a given condition for a given apparatus. (Table IV.)

The principal points to be noted regarding this experiment are:

1. The respiratory intensity which was about the same at the beginning of the experiment as shown in Table I, rose sharply with the onset of germination, but fell somewhat after germination, with the second period at the excessively high temperature of 30° C.

2. The respiratory quotient was high, as in the previous experiments, during incipient germination. The value of the respiratory quotient in this case reached 1.2, recalling Gerber's observations.[5]

3. The respiratory quotient fell in value as respiratory intensity increased with advancing germination. The lowest value of the respiratory quotient, as well as the figures expressing respiratory intensity during the same period, are here almost identical with those for the period of greatest respiratory intensity in Table I.

[5] See discussion on page 119

TABLE IV.—*Respiration of Newtown Pippin seeds from cold-stored fruit. Seeds not germinated after incubation at 5° to 10° C. for 13 to 18 days*

Period.	Temperature.	Description of seeds.	Gaseous exchanges. (Mgm. per gm. dry weight per day.)		CO_2/O_2 (volume).
			Oxygen consumed.	CO_2 produced.	
Hours.	° C.				
45	19	Several incipient germinations at end of period.	0.95	1.58	1.20
		Duplicate lots	.95	1.56	1.19
46	19	54 per cent germinating by end of period. Roots reddish.	.97	1.28	.95
46	19	A. Not germinated; 50 per cent germinating by end of period.	1.49	1.70	.83
		B. Germinating at beginning of period	1.94	2.19	.81
21	30	A. Not germinated; 44 per cent germinating by end of period.	1.80	2.78	1.11
		B. Germinated at beginning of period; slow growth.	11.15	10.00	.65
46	30	A. Same seeds as previous period; 52 per cent germinating by end of period.	2.01	2.56	.92
		B. Same seeds as previous period	6.13	5.97	.70

FOURTH EXPERIMENT.—Newtown Pippins. Cold stored in fruit until May 7. Seeds removed and incubated a few days at 5° to 10° C., then 12 days at 25°. Many germinated at 25°. Respiration experiment with those not yet germinated. Twenty-five seeds in each lot at first. Some discarded during the experiment on account of germination or to leave a convenient number in a given condition for a given apparatus. Experiment begun May 27, 1919. (Table V.)

The principal points to be noticed in this experiment are:

1. Respiratory intensity was lower during the first period than in the previous experiment with similar seeds previously incubated only in the ice box. This difference in resipratory intensity between the seeds used in the two experiments is correlated on the one hand with a possilbe assumption of a condition of secondary dormancy by the seeds at 25° C., and on the other hand with a known increase in metabolic activity during the last stages of after-ripening at the lower temperature. To be sure the selective effect of germination during the previous incubation would leave only the less active seeds to be used in the respiration experiments. But this factor could not account for the difference observed. Of the seeds originally put to germinate, nearly twice as large a percentage of those incubated at 5° to 10°, had germinated previous to the respiration experiment as was the case with those incubated at 25°. The selection resulting from germination would, therefore, of itself tend toward a lower rather than a higher respiratory activity in the seeds used in the previous experiment as compared with those used in the present experiment. Since the two experiments were begun at the same time, and conducted simultaneously throughout, there can be no difference due primarily to the mere lapse of time.

TABLE V.—*Respiration of Newtown Pippin seeds, not germinated after cold storage in the fruit to May 7, incubation at 5° to 10° C. for a few days and at 25° for 12 days*

Period.	Temperature.	Condition of seeds.	Gaseous exchanges. (mgm. per gm., dry weight, per day.)		CO_2/O_2 (volume).
			Oxygen consumed.	CO_2 produced.	
Hours.	° C.				
45	19	Dormant	0.80	0.77	0.70
46	19	A. 12 per cent just beginning to germinate at end of period	.85	.83	.72
		B. 24 per cent germinating at end of period; longest root 6 mm	1.45	1.42	.71
46	19	A. Not germinated; 16 per cent germinating at end of period	1.82	2.25	.89
		B. All germinated at beginning of period; fairly active growth; healthy	7.54	6.62	.63
21	30	A. Not germinated; 10 per cent germinating at end of period	1.45	1.71	.85
		B. Same seeds as B in preceding period; little new growth; roots curled and reddish	13.4	11.3	.61
46	30	A. Same seeds as in preceding period; 15 per cent germinating and 5 per cent moldy by end of period	1.36	1.53	.81
		B. Same seeds as B in preceding period; growth slow	9.69	8.55	.64

Furthermore, contemporaneous germination tests showed that very little germination occurred at 25° C. after the first 12 days, while at 5° to 10°, or a little above, germination continued slowly and was nearly complete within the next two weeks. There was therefore a progressive change going on in each case, tending to a condition of dormancy at 25°, and to complete germination at the lower temperature. The impoverishment of the seeds used in this experiment as a result of relatively high respiratory intensity during the previous period at 25°, and especially the relatively high respiratory quotient no doubt obtaining at that temperature on the one hand, and the low respiratory intensity and storage of oxygen (low respiratory quotient) characteristic of the lower temperature on the other hand, are no doubt related to the comparative behavior, both as to respiration and as to germination, when the seeds used in the two experiments were brought to the same intermediate temperature of 19°.

2. The respiratory intensity increased rapidly with advancing germination, but fell off during the second period at the excessively high temperature of 30° C.

3. The respiratory quotient corresponded to complete oxidation of the fats (0.70) at the beginning of the experiment, rose very slightly during the earliest emergence of radicles and much more during later incipient germinations, and fell markedly in later stages of germination in spite of the high temperature (30° C.), which gives a high respiratory quotient in dormant seeds.

FIFTH EXPERIMENT.—A. Seeds remaining in lot A of previous experiment of which this experiment is a continuation; B, 25 York Imperial seeds not germinated after cold storage in the fruit to February 5, 1919, removed from fruit February 15, and incubation at 5° to 10° C., four days, and at 25° 3½ months. This half of the experiment was begun May 27, 1919. (Table VI.)

TABLE VI.—*Respiration of Newtown Pippin and York Imperial seeds dormant after cold storage in the fruit and incubation at 25° C.*

Period.	Temperature.	Gaseous exchanges (mgm. per gm. dry weight) per day.				CO_2/O_2 (volume).	
		Oxygen consumed.		CO_2 produced.			
		A[1]	B[2]	A[1]	B[2]	A[1]	B[2]
Hours.	° *C.*						
45	19	.....	0.80	.....	0.81	.....	0.73
46	19	.....	.93	.....	.91	.....	.71
46	19	.....	.97	.....	.94	.....	.71
21	30	.....	1.56	.....	1.80	.....	.83
46	30	.....	1.19	.....	1.18	.....	.72
45	30	1.36	1.11	1.27	1.03	0.68	.67
43	19	.96	1.01	.78	.78	.59	.56
45	19	.85	.90	.91	.80	.77	.64
47	19	.81	.84	.86	.69	.77	.59
25	30	1.57	1.33	1.59	1.25	.73	.68
48	30	1.06	1.00	1.02	1.02	.81	.74
48	19	.93	.94	.90	.78	.70	.60
118	19	.59	.63	.59	.57	.72	.65
114	10	.60	.61	.51	.49	.61	.57
121	10	.62	.64	.51	.54	.59	.58
118	10	.62	.65	53	.46	.62	.50
194	0	.28	.30	.14	.16	.37	.39
140	13	.62	.65	.59	.59	.70	.66
49	30	1.35	1.25	1.51	1.47	.81	.85
			ALL OUTER COATS REMOVED[3]				
20	30	5.22	4.54	5.05	4.14	.70	.66
INNER COATS REMOVED[3]			ALL GERMINATING BY END OF PERIOD				
43	19	9.56	7.19	9.53	6.99	.72	.70

[1] Lot A, Newtown Pippin seeds dormant after cold storage in fruit, incubation at 5° to 10° C for a few days and then 25° for 12 days, and previous respiration experiment at 19° and 30° for 9 days.
[2] Lot B, York Imperial seeds dormant after cold storage in fruit, incubation at 5° to 10° C. for 4 days and at 25° for 3½ months.
[3] See footnote on page 118.

Table VI gives the data for the respiratory exchanges. None of these seeds germinated until the last period of the experiment after removal of the inner coats. All results were calculated on the basis of the dry weight of the material used in each period, whether this included coat structures or not.

1. The respiratory intensity was generally much lower than that of the dormant seeds with outer coats removed, which were used in experiment 2, but on account of the method used of computing the results in relation to the material actually used, the differences in relation to the actively respiring portions were probably not as large as would appear from a comparison of the two tables.

During the last long period (118 hours) at 19° C. the respiratory intensity was so reduced that for the whole period the average intensity was about the same as for the subsequent periods at 10° and for a later period at 13°. It is not possible to state the reason for such a fall in respiratory intensity during this period. It can not be the direct result of greater reduction in oxygen pressure or greater increase in CO_2 pressure than in previous periods, since the actual amounts of gaseous

exchange were scarcely greater during this period than during some previous periods at 30° C.

2. Respiratory quotients were generally about the same as in experiment 2, but rather irregular, especially with lot A in the first few periods following the preceding experiment in which many germinated; except for these irregularities quotients rose and fell with rise and fall of temperature, and they became very low at 0° C.; the respiratory quotients were higher for lot A than for lot B, probably for the reason suggested in experiment 2 (see page 120).

3. Removal of the outer coats greatly increased respiratory intensity but decreased respiratory quotients as if rendering oxygen more available. If this is the correct explanation, then it must be that the inner coats are readily permeable to oxygen in aqueous solution, and this must hold, too, for CO_2. Such an assumption would explain the greater respiratory intensity of the seeds used in experiment 2, as compared with those used in experiment 5, and the sporadic germination of a small percentage of the seeds used in experiment 2 during the course of the experiment would then appear as probably the result of such increase in permeability and consequent increase in respiratory intensity. With prolonged exposure of the inner coats to the air, as in the case of experiment 2, they become somewhat brownish and perhaps less permeable.

4. With removal of the inner coats, the seeds all germinated with greatly increased respiratory intensity and little change in respiratory quotients. The air in the apparatuses at the end of this period contained about 1 per cent oxygen.

Table VII gives the temperature coefficients for oxygen consumption and CO_2 production for consecutive periods with all temperature intervals used in experiment 5.

TABLE VII.—*Temperature coefficients for respiration of dormant intact apple seeds*

Temperature interval.	Q_{10} for oxygen consumpton.		Q_{10} for CO_2 production.	
	A [1]	B [2]	A [1]	B [2]
°C.				
19 to 30	.....	1.54	.....	1.80
30 to 19	1.37	1.09	1.56	1.29
19 to 30	1.83	1.52	1.75	1.71
30 to 19	1.13	1.05	1.30	1.28
19 to 10	.99	1.02	1.18	1.19
10 to 0	2.25	2.17	3.79	2.79
0 to 13	1.86	1.80	3.04	2.69
13 to 30	1.59	1.48	1.73	1.71

[1] Lot A, Newtown Pippin seeds dormant after cold storage in fruit, incubation at 5° to 10° C. for a few days and then 25° for 12 days, and previous respiration experiment at 19° and 30° for 9 days.
[2] Lot B, York Imperial seeds dormant after cold storage in fruit, incubation at 5° to 10° C. for 4 days and at 25° for 3½ months.

As in experiment 2, the values for Q_{10} are greater for CO_2 production than for oxygen consumption, greater for the lot which I have supposed to be richer in the more easily oxidizable materials (A) than for the other lot (B), greater when changing from 19° to 30° C. than when the reverse change was made, and greater at low temperatures than at higher temperatures.

As a result of the very low respiratory intensity during the 118-hour period at 19° C., the temperature coefficients from this temperature to 10° appeared very low.

From Table VII and the previous table of temperature coefficients (Table III) it is evident that the values of Q_{10} for the respiration of seeds for any given temperature interval may depend upon a number of different factors, which have not as yet been worked out, but among which the physiological condition of the seed and its previous treatment are important.

It is impossible to draw reliable conclusions as to the nature of the complex of processes which constitute respiration from any one set of temperature coefficients. Nevertheless, it seems entirely safe to say that different steps in the complex are differently affected by temperature changes—in other words, have differential temperature coefficients—and that these differences are reflected in the physiological condition of the living embryo can scarcely be doubted.

IMPORTANCE OF STUDYING OXYGEN CONSUMPTION AND CO_2 PRODUCTION IN THEIR RELATION TO EACH OTHER

A great many, though by no means all, investigators of respiration have confined their attention to either oxygen consumption or CO_2 production to the exclusion of the other, and frequently the apparatus used has rendered the study of the other impossible. Different investigators have been led to consider of paramount importance one or the other of these two evidences of respiratory activity. Mayer (*18*), for instance, studied only oxygen consumption because this was supposed to correspond much more nearly to the amount of heat produced by respiration than does CO_2 production. Much of the oxygen consumed does not reappear as CO_2 but is built into the plant tissues. In this case CO_2 is not the end product of the oxidation. On the other hand, he points out that CO_2 is given off by the reduction of oxygen-rich mineral acids, quite independent of true respiration. Tashiro (*22*) and, following him, Gurjar (*10*) emphasized CO_2 production as "an accurate index of respiratory activity."

It would seem from the data in this paper that neither oxygen consumption nor CO_2 production can be considered as "an accurate index of respiratory activity." Both depend upon external as well as internal conditions, which affect the two differently, so that neither alone gives a complete picture, much less a satisfactory understanding of respiratory exchanges. It seems certain, furthermore, that following oxygen intake and CO_2 output, in their relation to each other and to external factors, will be useful in pointing the way to fruitful investigations in other fields of plant physiology.

SUMMARY

(1) The respiratory intensity of dormant apple seeds is low. The respiratory intensity of seeds capable of germination is higher and becomes very high with advancing germination but soon falls somewhat if the germinated seeds are kept at too high a temperature (30°).

(2) Removal of the outer seed coats or of both seed coats increases respiratory intensity and accelerates germination.

(3) The respiratory quotient of dormant apple seeds at ordinary temperatures (19° C.) corresponds to complete oxidation of fats (0.70) or to only slight increase in sugars.

(4) The respiratory quotient increases with increase in temperature, causing impoverishment in easily oxidizable substances and possibly indicating oxygen deficiency in the respiring tissues.

(5) The respiratory quotient decreases with decrease in temperature, indicating a storage of oxygen which becomes very considerable at 10° and 0° C. and probably leads to increase in acids and sugars.

(6) Correlated with a relatively high rate of oxidation at high temperatures is a tendency for the seeds to become more dormant; with storage of oxygen at low temperatures they slowly become capable of germination.

(7) The respiratory quotient becomes low (about 0.60) with advancing germination, indicating the rapid transformation of fats and accumulation of sugars. Preceding this fall in respiratory quotient there is frequently a brief initial rise (to a maximum of 1.2), which indicates the breaking up of oxygen-rich bodies (probably organic acids) much more rapidly than these bodies are replaced and may be associated with a temporary deficiency of oxygen in the respiring tissues.

(8) The temperature coefficients for the respiratory exchanges of dormant apple seeds are greater for seeds previously incubated at medium temperatures (20° C.) than for seeds previously incubated at higher temperatures, greater for CO_2 production than for oxygen consumption, greater when changing from a medium temperature (19° C.) to a higher temperature (30° C.) than when the reverse change is made, and greater at low temperatures than at high temperatures.

(9) Temperature changes, at least temporary elevation of the temperature (to 30° C.), may exert a stimulating effect on the respiration of dormant apple seeds, but the evidence is not conclusive.

(10) Respiratory intensity, respiratory quotients, and temperature coefficients are affected by the previous treatment of the seeds, being higher after treatment which tends toward after-ripening, and lower after treatment which induces deeper dormancy.

(11) Temperature coefficients are different for different steps in the oxidative processes which constitute respiration, and these differences are related to the different temperature effects upon the physiological condition of the living embryo.

(12) In order to gain an understanding of respiratory processes it is necessary to study oxygen consumption and CO_2 production in their relation to each other.

LITERATURE CITED

(1) APPLEMAN, Charles O.
1911. PHYSIOLOGICAL BEHAVIOR OF ENZYMES AND CARBOHYDRATE TRANSFORMATIONS IN AFTER-RIPENING OF THE POTATO TUBER. *In* Bot. Gaz., v. 52, no. 4, p. 306–315. Literature cited, p. 315.

(2) ATWOOD, W. M.
1914. A PHYSIOLOGICAL STUDY OF THE GERMINATION OF AVENA FATUA. *In* Bot. Gaz., v. 57, no. 5, p. 386–414, 13 fig. Literature cited, p. 410–414.

(3) BLANC, L.
1916. RECHERCHES EXPÉRIMENTALES SUR L'INFLUENCE DES VARIATIONS DE TEMPÉRATURE SUR LA RESPIRATION DES PLANTES. *In* Rev. Gén. Bot., t. 28, no. 327, p. 65–79.

(4) BONNIER, Gaston, and MANGIN, L.
1884. SUR LES VARIATIONS DE LA RESPIRATION DES GRAINES GERMANT AVEC LE DÉVELOPPEMENT. *In* Bul. Soc. Bot. France, t. 31 (sér. 2, t. 6), p. 306–309.

(5) BRANNON, M. A.
1913. OSMOTIC PRESSURE IN POTATOES. *In* Bot. Gaz., v. 56, no. 5, p. 433–438, 4 fig.

(6) CROCKER, William, and HARRINGTON, George T.
1918. CATALASE AND OXIDASE CONTENT OF SEEDS IN RELATION TO THEIR DORMANCY, AGE, VITALITY, AND RESPIRATION. *In* Jour. Agr. Research, v. 15, no. 3, p. 137–174, 3 fig. Literature cited, p. 173–174.

(7) ECKERSON, Sophia.
1913. A PHYSIOLOGICAL AND CHEMICAL STUDY OF AFTER-RIPENING. *In* Bot. Gaz., v. 55, no. 4, p. 286–299. Literature cited, p. 297–299.

(8) GERBER, C.
1900. ÉTUDE COMPARÉE DE LA RESPIRATION DES GRAINES OLÉAGINEUSES PENDANT LEUR DÉVELOPPEMENT ET PENDANT LEUR GERMINATION. RELATION ENTRE CETTE RESPIRATION ET LES RÉACTIONS CHIMIQUES DONT LA GRAINE EST LA SIÈGE. *In* Actes 1er Cong. Internat. Bot. Paris, 1900, p. 59–101.

(9) GODLEWSKI, Emil.
1882. BEITRÄGE ZUR KENNTNISS DER PFLANZENATHMUNG. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 13, Heft 3, p. 491–543, 1 fig.

(10) GURJAR, A. M.
1917. THE ADAPTATION OF TRUOG'S METHOD FOR THE DETERMINATION OF CARBON DIOXIDE TO PLANT RESPIRATION STUDIES. *In* Plant World, v. 20, no. 9, p. 288–293, 1 fig.

(11) HARRINGTON, George T., and CRACKER, William.
1922. A NEW AND EFFICIENT RESPIROMETER FOR SEEDS AND OTHER SMALL OBJECTS: DIRECTIONS FOR ITS USE. *In* Jour. Agr. Research, v. 23, no. 2, p. 101–116, 2 fig., 1 pl. Literature cited, p. 113–115.

(12) IRWIN, Marian.
1919. COMPARATIVE STUDIES ON RESPIRATION. VI. INCREASED PRODUCTION OF CARBON DIOXIDE ACCOMPANIED BY DECREASE OF ACIDITY. *In* Jour. Gen. Physiol., v. 1, no. 4, p. 399–403, 2 fig.

(13) IVANOW, Sergius L.
1913. DER EINFLUSS VON OELVORRÄTEN DER SAMEN UND DER TEMPERATUR AUF DEN RESPIRATIONSCOEFFICIENT $\frac{CO_2}{O_2}$. *In* Zhur. Opytn. Agron. (Russ. Jour. Exp. Landw.), t. 14, kniga 1, p. 75–87. *In* Russian. German résumé, p. 87.

(14) JONES, H. A.
1920. PHYSIOLOGICAL STUDY OF MAPLE SEEDS. *In* Bot. Gaz., v. 69, no. 2, p. 127–152, 2 fig. Literature cited, p. 151–152.

(15) KOHL, F. G.
1910. ÜBER DAS WESEN DER ALKOHOLGÄRUNG. *In* Bot. Centralbl., Beihefte, Bd. 25, Abt. 1, Heft 2, p. 115–126.

(16) LUND, J. E.
1918. QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION. IV. THE RATES OF CO_2 PRODUCTION BY STARVED AND FED PARAMŒCIA, AND THEIR POSSIBLE RELATION TO THE RATES OF OXIDATION IN THE UNFERTILIZED AND FERTILIZED SEA URCHINS EGG. *In* Amer. Jour. Physiol., v. 47, no. 3, p. 318–327. Bibliography, p. 327.

(17) ———
1920. THE RELATION OF THE CONCENTRATION OF OXYGEN TO THE RATE OF RESPIRATORY METABOLISM IN PLANARIA. (Abstract.) *In* Anat. Rec., v. 17, no. 5, p. 342–343.

(18) MAYER, Adolph.
1875. UEBER DEN VERLAUF DER ATHMUNG BEIM KEIMENDEN WEIZEN. *In* Landw. Vers. Stat., Bd. 18, p. 245–279.

(19) OSTERHOUT, W. J. V.
1918. A METHOD OF STUDYING RESPIRATION. *In* Jour. Gen. Physiol., v. 1, no. 1, p. 17–22, 1 fig.

(20) PALLADINE, W.
1899. INFLUENCE DES CHANGEMENTS DE TEMPÉRATURE SUR LA RESPIRATION DES PLANTES. *In* Rev. Gén. Bot., t. 11, no. 127, p. 241–257.

(21) ROSE, R. C.
1919. AFTER-RIPENING AND GERMINATION OF SEEDS OF TILIA, SAMBUCUS, AND RUBUS. *In* Bot. Gaz., v. 67, no. 4, p. 281–308. Literature cited, p. 307–308.

(22) TASHIRO, Shiro.
1913. A NEW METHOD AND APPARATUS FOR THE ESTIMATION OF EXCEEDINGLY MINUTE QUANTITIES OF CARBON DIOXIDE. *In* Amer. Jour. Physiol., v. 32, no. 2, p. 137–145, 2 fig.

(23) ZIEGENBEIN, Ernst.
1893. UNTERSUCHUNGEN ÜBER DEN STOFFWECHSEL UND DIE ATHMUNG KEIMENDER KARTOFFELKNOLLEN SOWIE ANDERER PFLANZEN. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 25, Heft 4, p. 563–606.